Risk assessment of biological treatment in inflammatory bowel disease & analysis of genetic susceptibility factors

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Risk assessment of biological treatment in inflammatory bowel disease & analysis of genetic susceptibility factors

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General introduction and aim of this thesis

Chapter 1

Epidemiology of inflammatory bowel disease

Inflammatory bowel disease (IBD) is an idiopathic chronic inflammation of the gastrointestinal tract and consists of two major types; Crohn's disease and ulcerative colitis. The incidence and prevalence of IBD varies worldwide.[1] Traditionally, the highest numbers are reported in Northern - and Western Europe and North America, but increasing incidence rates have been reported from previous low incidence and prevalence areas as Eastern Europe, South America, Asia and the Pacific region.[2] An estimated 50,000 to 68,000 new cases of ulcerative colitis and 23,000 to 41,000 new cases of Crohn's disease are diagnosed annually throughout Europe. Collectively approximately 3.6-3.7 million patients suffer from IBD worldwide.[1, 3]

The increasing trend in incidence and prevalence of IBD over the last decades and its emergence in developing countries suggests a role of the Western lifestyle in the pathogenesis.[4] Moreover, epidemiologic data from migrant populations show that the incidence and prevalence of IBD in non-white ethnic groups increases after migrating to another geographical area, indicating that genetic and environmental factors interact to determine risk for IBD early in life.[1]

Clinical and pathological aspects of inflammatory bowel disease

The peak age of onset of ulcerative colitis and Crohn's disease is between 15 and 30 years, while a second peak occurs between 60-80 years.[5] Although both ulcerative colitis and Crohn's disease are chronic inflammatory disorders of the gastrointestinal tract, there is a distinct clinical and pathological presentation.

From a pathological perspective Crohn's disease is characterized by transmural inflammation involving the entire gut wall, granuloma formation and a characteristic cobblestone appearance due to an intermittent pattern of diseased and healthy tissue. It can affect any part of the gastrointestinal tract. The most commonly affected site is the terminal ileum.[5] The disease course is typified by a sequence of flare-up episodes and remissions of varying durations, and clinical patterns vary according to disease location and disease behavior (penetrating, stricturing, and nonpenetrating/nonstricturing or inflammatory).[1, 6] Clinically, key features of Crohn's disease are (mild) diarrhea, (low-grade) fever, abdominal mass, signs of malnutrition, weight loss and episodes of right lower quadrant abdominal pain.[5, 7] Progression of anatomic damage leads to development of penetrating or stricturing complications such as strictures, inflammatory masses, abscesses, and fistulae.[1] The large majority of patients with Crohn's disease (70-80%) will require intestinal surgery within a disease course of 20 years.[1] Even with the recent developments in medical and surgical treatment options, there appears to a slight but significant higher mortality in patients with Crohn's disease.[8]

Ulcerative colitis is characterized by inflammation limited to the mucosa of the colon, usually involving the rectum with proximal extending. The clinical course is characterized by episodes of active and inactive disease, a pattern observed in 80-90% of the patients.[6]

Since the mucosa continues to be destroyed, subsequent development of frailty vascular granulation tissue and electrolyte and water loss through the mucosa occurs. Therefore, the major symptoms of ulcerative colitis are rectal bleeding and diarrhea. Other symptoms are crampy abdominal pain, passage of mucus and tenesmus. The severity of symptoms correlates with the extent of the disease.[5]
Apart from the predominant intestinal manifestations, approximately one-third of the patients with IBD develop extra-intestinal manifestations, including peripheral arthritis, erythema nodosum, aphthous ulcers, pyoderma gangrenosum, uveitis, spondylarthropathy, and primary sclerosing cholangitis.[9, 10]

Pathogenesis

Despite intensive study, the etiology and pathogenesis of IBD is currently still unclear. It is commonly thought that IBD is caused by an inappropriate and continuing inflammatory response to gut microbiota (commensal bacteria) in genetically susceptible individuals.[11, 12] Thus it involves interactions among immune, environmental and genetic factors, resulting in the induction of inflammation, subsequent development of mucosal lesions and repair.[13]

Genetic factors

Twin studies have initially highlighted the importance of genetic involvement in the pathogenesis of IBD and showed that genetic susceptibility is more pronounced in Crohn's disease compared to ulcerative colitis.[14, 15] In the past decades, several susceptibility loci for IBD have been identified. More recently genome wide association studies (GWAS) have identified at least 71 risk loci in Crohn's disease and 47 risk loci in ulcerative colitis, including 28 that are shared between both disease entities.[11, 16, 17] Many of the mutations found were in genes encoding recognition, processing and killing of microorganisms and the regulation of immune processes.[13] The findings highlighted pathways which were identified before trough immunological studies (IL-23, TH17 cells) as well as new pathways such as autophagy (the *ATG16L1* and *IRGM* genes),[18, 19] the process in which cellular contents can be degraded by lysosomes to be recycled in order to provide nutrients.[20] This mechanism also plays crucial roles in the immune system for elimination of potentially deleterious proteins, organelles and pathogens.[21]
Most of the alleles identified by GWAS are relatively common (allele frequencies >5%) and have modest or low effects with odds ratios < 1.5.[22] As a single genetic risk factor, only *nucleotide binding oligomerization domain protein 2 (NOD2)* has a meaningful contribution to Crohn's disease risk given that homozygosity for *NOD2* mutations is associated with a > 20-fold increased risk for Crohn's disease in Caucasians.[23, 24] The contribution of *NOD2* mutations to the development of Crohn's disease underlines the importance of the intestinal microbiota and innate mucosal defense in the pathogenesis of IBD. NOD2 acts as an intracellular pattern recognition receptor (PRR) recognizing muramyl dipeptide (MDP) which is a conserved structure in bacterial peptidoglycans.[25] Genetic variants in *NOD2* causes a deficiency to detect

MDP.[26] Moreover, NOD2 is required for the expression of a subgroup of intestinal anti-microbial peptides and thus critical for regulation of bacterial immunity within the intestine.[27]

The role of microbiota

The role of luminal bacterial contents in IBD is well-recognized, based on several observations.[28] (I) Crohn's disease and ulcerative colitis preferentially occur in the colon and distal ileum, which contain the highest intestinal bacterial concentrations; bacteria and fungi increase in both concentration and complexity from the proximal gastric and duodenal population of 10^2 – 10^3 aerobic organisms/gram luminal contents to 10^{11} – 10^{12} predominantly anaerobic bacteria/ gram in the cecum and colon.[12] (II) Patients with Crohn's disease and their unaffected relatives have a different composition of the intestinal microbiota, and patients with IBD even have higher concentrations of mucosal bacteria compared with healthy controls.[29, 30] (III) These observations accord with evidence from experimental animal models in which the animals are susceptible for developing intestinal inflammation. In example, the HLA-B27 transgenic rat, a well known model of chronic intestinal inflammation, develops spontaneous colitis in the presence of commensal intestinal bacteria, but remains disease free in the germfree state.[31, 32] (IV) In clinical practice, diversion of fecal stream can prevent and treat Crohn's disease and recurrence of inflammation is observed on restoration or exposure to faecal material.[33-35]

Intestinal immune system

The intestinal immune system represents a complex network of different lymphoid and non-lymphoid cell populations and humoral factors.[36] In IBD, the immune defense against intestinal microbes fails at two levels: the epithelial mucosal barrier, which is the first line of defense of the mucosal immune system is leaky and impaired,[37-40] and the innate and acquired host immune responses towards the intestinal microbiota are altered.[41] Traditionally, Crohn's disease has been characterized as a T helper (T_H) 1 disease by producing large quantities of interferon-γ (IFN-γ) and tumor necrosis factor α (TNF-α) while ulcerative colitis seemed to exhibit a T_H2-type cytokine profile by producing IL-4 and IL-13.[42, 43] Recently, a third class of CD4+ positive cells producing IL-17 and therefore called 'Th17 cells' have been identified, which are thought to contribute to the pathogenesis of IBD.[43-45] Expression of these cells has been observed in the gut of patients with Crohn's disease[46] and abundant production of IL-17 has been observed in inflamed mucosa of IBD patients.[47]

Environmental factors

Among the environmental factors possibly involved in the pathogenesis of IBD, smoking clearly affects the risk for developing Crohn's disease.[1] On the contrary, smoking reduces the risk for developing ulcerative colitis.[48]

Treatment

Since the etiology of inflammatory bowel disease is still unclear, causative therapy is not available. IBD is a lifelong disorder and medical and surgical therapy should therefore be focused on long term outcomes with the primary treatment goal to induce and to maintain remission in a safe and efficacious way, and restore normal bowel function.[6, 49]

Conventional agents used in the medical therapy of inflammatory bowel disease consist of three different subgroups of drugs, derivates of 5-aminosalicylic acid (5-ASA), corticosteroids, and immunomodulatory agents such as azathioprine, 6-mercaptopurine, methotrexate and cyclosporine. Increasing knowledge on the central role of tumor necrosis factor α (TNF-α) in the inflammation present in IBD,[50-54] led to the development and use of (monoclonal) antibodies targeting this cytokine, including infliximab and adalimumab, in the treatment of IBD.[55-59]

In general, therapeutic recommendations for Crohn's disease depend on age, disease location, disease severity, and disease-associated complications. A step up approach is generally recommended in which more powerful therapies are initiated as the severity of disease and refractoriness to therapy increases, as depicted in figure 1.1.[60-62] Treatment is initiated with agents as 5-ASA derivates, budesonide, or systemic corticosteroids, followed by continuous immunosuppressive regime. Following this approach, patients who are unable to obtain or remain in remission despite adequate treatment with immunosuppressive drugs, patients with intolerance to conventional treatment (i.e. corticosteroids and immunosuppressants) and patients with enterocutaneous fistulas not responding to conventional therapies, are eligible for treatment with anti TNF-α therapies.[49]

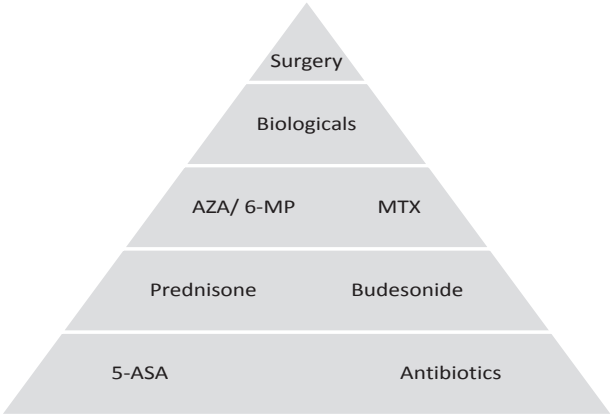

Figure 1.1 Step up approach depicted (adapted from ref 63)

The traditional step up approach has been challenged however following a study by D'Haens *et al*.[64] This study compared the effectiveness of the traditional strategy with a top down approach that combined immunosuppression of infliximab and azathioprine as the initiating treatment in active Crohn's disease patients naïve to glucocorticoids, antimetabolites, or infliximab. They demonstrated superiority of the top down approach for early induction of remission and reduction of corticosteroid use in patients who had been recently diagnosed with Crohn's disease. Furthermore, a recent study showed that infliximab monotherapy or infliximab plus azathioprine combination therapy is superior to azathioprine alone in immunosuppressant naive patients to maintain corticosteroid free remission and obtain mucosal healing.[65] This indicates, apart from other studies, that an accelerated use of immunosuppressants and anti TNF therapy will induce and maintain remission, reduce steroid use and promote mucosal healing. Mucosal healing is associated with better long-term disease outcomes (less hospitalization and abdominal surgery, reduced relapse rate, steroid free remission).[66-68] As been pointed out in a recent review, managing IBD can be compared with a dance with familiar steps (i.e. mesalamine, steroids, immunomodulators, or anti-TNF therapy and surgery) but the timing and sequence of the steps creates the dance.[69]

Apart from pharmacological, non pharmacological interventions can play an important role in the treatment of IBD. Smoking is a risk factor for disease relapse after medically- or surgically-induced remission in patients with Crohn's disease. It is also associated with the need for higher doses of corticosteroids and immunosuppressants, and should therefore be discouraged in patients.[70-73] Moreover, one year of smoking cessation leads to a more benign Crohn's disease course with lower relapse rates.[74]

Although the therapeutic benefits of medical therapy in IBD are of major importance, it is frequently offset by numerous side effects, including bone marrow suppression (methotrexate, thiopurines), hepatitis and pancreatitis (5-ASA derivates, methotrexate, thiopurines), fluid retention, fat redistribution, hypertension, hyperglycaemia, psycho-neurological disturbances, cataracts, adrenal suppression, growth failure in children, and osteonecrosis (corticosteroids).[75] Therefore, effects and adverse effects should be balanced while determining the appropriateness of medical therapy in IBD.

Outline of this thesis

The main objective of this thesis was to evaluate whether the use of infliximab is an adequate treatment of inflammatory bowel disease (IBD) in view of both effects and safety .

Specific themes were studied, aiming:

1. To evaluate the occurrence of serious events after the onset of infliximab treatment in patients with IBD, with a mean follow-up of 9 years (Chapter 2A & 2B).
2. To evaluate the necessity of monitoring vital signs during infusion therapy with infliximab (Chapter 2C).
3. To identify similarities and differences within international, national and local guidelines and additional consensus statements from the medical specialties currently using infliximab (Chapter 3), regarding:
    a. Indications for infliximab
    b. Dosage for initial and maintenance therapy
    c. Monitoring of vital signs during infusion with infliximab
    d. Synergetic effects with concomitant medication use
4. To evaluate the direct hepatotoxicity of infliximab as compared to conventional therapies used in the treatment of IBD (Chapter 4).

A subsequent objective was to identify genetical susceptibility factors which might be associated with the development of inflammatory bowel disease.

Specific themes with regards to this objective were:

1. To study the relationship between a polymorphism in *UGT1A1* and drug side effects in patients with IBD (Chapter 5).
2. To test the hypothesis that the functional *DECTIN-1* c.714T>G (p.Y238X) polymorphism is associated with lower disease susceptibility or severity in IBD and to investigate the level of dectin-1 expression in inflamed and non-inflamed colon tissue of IBD patients (Chapter 6).
3. To investigate the possible modulating effect of the functional *COX-2* polymorphisms -1195 A→G and -765G→C on the risk for development of IBD in a Dutch population (Chapter 7).

References

1. Cosnes J, Gower-Rousseau C, Seksik P, et al Epidemiology and Natural History of Inflammatory Bowel Diseases. Gastroenterology 2011;140:1785-1794.
2. Lakatos PL. Recent trends in the epidemiology of inflammatory bowel diseases: Up or down? World J Gastroenterol 2006;12:6102-6108.
3. Loftus EV. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. Gastroenterology 2004;126:1504-1517.
4. Gibson PR, Shepherd SJ. Personal view: food for thought - western lifestyle and susceptibility to Crohn's disease. The FODMAP hypothesis. Aliment Pharmacol Ther 2005;21:1399-1409.
5. Friedman S, Blumberg RS. Inflammatory Bowel Disease. In: Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, and Loscalzo J, eds. Harrison's Principles of Internal Medicine. 18 ed. McGraw Hill, 2011.
6. Blonski W, Buchner AM, Lichtenstein GR. Inflammatory bowel disease therapy: current state-of-the-art. Curr Opin Gastroenterol 2011;27:346-357.
7. Podolsky DK. Inflammatory bowel disease. N Engl J Med 2002;347:417-429.
8. Peyrin-Biroulet L, Loftus EV, Colombel JF, et al Long-term Complications, Extraintestinal Manifestations, and Mortality in Adult Crohn's Disease in Population-Based Cohorts. Inflamm Bowel Dis 2011;17:471-478.
9. Rothfuss KS, Stange EF, Henrlinger KR. Extraintestinal manifestations and complications in inflammatory bowel diseases. World J Gastroenterol 2006;12:4819-4831.
10. Vavricka SR, Brun L, Ballabeni P, et al. Frequency and Risk Factors for Extraintestinal Manifestations in the Swiss Inflammatory Bowel Disease Cohort. American Journal of Gastroenterology 2011;106:110-119.
11. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature 2011;474:307-317.
12. Sartor RB. Microbial influences in inflammatory bowel diseases. Gastroenterology 2008;134:577-594.
13. Tlaskalova-Hogenova H, Stepankova R, Kozakova H, et al. The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. Cell Mol Immunol 2011;8:110-120.
14. Halfvarson J, Bodin L, Tysk C, et al. Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. Gastroenterology 2003;124:1767-1773.
15. Tysk C, Lindberg E, Jarnerot G, et al. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. Gut 1988;29:990-996.
16. Franke A, McGovern DP, Barrett JC, et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nat Genet 2010;42:1118-1125.
17. Anderson CA, Boucher G, Lees CW, et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. Nat Genet 2011;43:246-252.
18. Parkes M, Barrett JC, Prescott NJ, et al. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. Nat Genet 2007;39:830-832.

19. Rioux JD, Xavier RJ, Taylor KD, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet 2007;39:596-604.
20. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. Annu Rev Immunol 2010;28:573-621.
21. Huett A, Goel G, Xavier RJ. A systems biology viewpoint on autophagy in health and disease. Curr Opin Gastroenterol 2010;26:302-309.
22. Cho JH, Brant SR. Recent insights into the genetics of inflammatory bowel disease. Gastroenterology 2011;140:1704-1712.
23. Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001;411:599-603.
24. Hampe J, Cuthbert A, Croucher PJ, et al. Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. Lancet 2001;357:1925-1928.
25. Girardin SE, Boneca IG, Viala J, et al. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 2003;278:8869-8872.
26. Inohara N, Ogura Y, Fontalba A, et al. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. J Biol Chem 2003;278:5509-5512.
27. Kobayashi KS, Chamaillard M, Ogura Y, et al. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 2005;307:731-734.
28. Ewaschuk JB, Dieleman LA. Probiotics and prebiotics in chronic inflammatory bowel diseases. World J Gastroenterol 2006;12:5941-5950.
29. Swidsinski A, Ladhoff A, Pernthaler A, et al. Mucosal flora in inflammatory bowel disease. Gastroenterology 2002;122:44-54.
30. Joossens M, Huys G, Cnockaert M, et al. Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives. Gut 2011;60:631-637.
31. Taurog JD, Richardson JA, Croft JT, et al. The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. J Exp Med 1994;180:2359-2364.
32. Hammer RE, Maika SD, Richardson JA, et al. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human beta 2m: an animal model of HLA-B27-associated human disorders. Cell 1990;63:1099-1112.
33. D'Haens GR, Geboes K, Peeters M, et al. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. Gastroenterology 1998;114:262-267.
34. Rutgeerts P, Goboes K, Peeters M, et al. Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. Lancet 1991;338:771-774.
35. Winslet MC, Allan A, Poxon V, et al. Faecal diversion for Crohn's colitis: a model to study the role of the faecal stream in the inflammatory process. Gut 1994;35:236-242.
36. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. Lancet 2007;369:1627-1640.
37. May GR, Sutherland LR, Meddings JB. Is small intestinal permeability really increased in relatives of patients with Crohn's disease? Gastroenterology 1993;104:1627-1632.
38. Breslin NP, Nash C, Hilsden RJ, et al. Intestinal permeability is increased in a proportion of spouses of patients with Crohn's disease. Am J Gastroenterol 2001;96:2934-2938.
39. Gerova VA, Stoynov SG, Katsarov DS, et al. Increased intestinal permeability in inflammatory bowel diseases assessed by iohexol test. World J Gastroenterol 2011;17:2211-2215.

40. Arrieta MC, Bistritz L, Meddings JB. Alterations in intestinal permeability. Gut 2006; 55:1512-1520.
41. Matricon J, Barnich N, Ardid D. Immunopathogenesis of inflammatory bowel disease. Self Nonself 2010;1:299-309.
42. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448:427-434.
43. Sarra M, Pallone F, MacDonald TT, et al. IL-23/IL-17 axis in IBD. Inflamm Bowel Dis 2010;16:1808-1813.
44. Harrington LE, Hatton RD, Mangan PR, et al. Interleukin 17-producing CD4(+) effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005;6:1123-1132.
45. Hue S, Ahern P, Buonocore S, Kullberg MC, et al. Interleukin-23 drives innate and T cell-mediated intestinal inflammation. J Exp Med 2006;203:2473-2483.
46. Annunziato F, Cosmi L, Santarlasci V, et al. Phenotypic and functional features of human Th17 cells. J Exp Med 2007;204:1849-1861.
47. Rovedatti L, Kudo T, Biancheri P, et al. Differential regulation of interleukin 17 and interferon gamma production in inflammatory bowel disease. Gut 2009;58:1629-1636.
48. van der Heide F, Dijkstra A, Weersma RK, et al. Effects of active and passive smoking on disease course of Crohn's disease and ulcerative colitis. Inflamm Bowel Dis 2009;15:1199-1207.
49. Danese S, Colombel JF, Reinisch W, et al. Review article: infliximab for Crohn's disease treatment--shifting therapeutic strategies after 10 years of clinical experience. Aliment Pharmacol Ther 2011;33:857-869.
50. Breese EJ, Michie CA, Nicholls SW, et al. Tumor necrosis factor alpha-producing cells in the intestinal mucosa of children with inflammatory bowel disease. Gastroenterology 1994;106:1455-1466.
51. MacDonald TT, Hutchings P, Choy MY, et al. Tumour necrosis factor-alpha and interferon-gamma production measured at the single cell level in normal and inflamed human intestine. Clin Exp Immunol 1990;81:301-305.
52. van Dullemen HM, van Deventer SJ, Hommes DW, et al. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). Gastroenterology 1995;109:129-135.
53. Papadakis KA, Targan SR. Role of cytokines in the pathogenesis of inflammatory bowel disease. Annu Rev Med 2000;51:289-298.
54. Murch SH, Lamkin VA, Savage MO, et al. Serum concentrations of tumour necrosis factor alpha in childhood chronic inflammatory bowel disease. Gut 1991;32:913-917.
55. Hanauer SB, Feagan BG, Lichtenstein GR, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet 2002;359:1541-1549.
56. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med 2005;353:2462-2476
57. Sandborn WJ, Feagan BG, Stoinov S, et al. Certolizumab pegol for the treatment of Crohn's disease. N Engl J Med 2007;357:228-238.
58. Afif W, Leighton JA, Hanauer SB, et al. Open-label study of adalimumab in patients with ulcerative colitis including those with prior loss of response or intolerance to infliximab. Inflamm Bowel Dis 2009;15:1302-1307.
59. Colombel JF, Sandborn WJ, Rutgeerts P, et al. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. Gastroenterology 2007;132:52-65.

60. Lichtenstein GR, Hanauer SB, Sandborn WJ. Management of Crohn's disease in adults. Am J Gastroenterol 2009;104:465-483.
61. Dignass A, van Assche G, Lindsay JO, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Current management. J Crohns Colitis 2010;4:28-62.
62. van Bodegraven AA, van Everdingen JJ, Dijkstra G, et al. [Guideline 'Diagnosis and treatment of inflammatory bowel disease in adults'. I. Diagnosis and treatment]. Ned Tijdschr Geneeskd 2010;154:A1899.
63. Panaccione R, Rutgeerts P, Sandborn WJ, et al. Review article: treatment algorithms to maximize remission and minimize corticosteroid dependence in patients with inflammatory bowel disease. Aliment Pharmacol Ther 2008;28:674-688.
64. D'Haens G, Baert F, van Assche G, et al. Early combined immunosuppression or conventional management in patients with newly diagnosed Crohn's disease: an open randomised trial. Lancet 2008;371:660-667.
65. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med 2010;362:1383-1395.
66. Colombel JF, Rutgeerts P, Reinisch W, et al. Early Mucosal Healing With Infliximab Is Associated With Improved Long-term Clinical Outcomes in Ulcerative Colitis. Gastroenterology 2011 [epub].
67. Baert F, Moortgat L, van Assche G, et al. Mucosal healing predicts sustained clinical remission in patients with early-stage Crohn's disease. Gastroenterology 2010;138:463-468.
68. Froslie KF, Jahnsen J, Moum BA, et al. Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort. Gastroenterology 2007;133:412-422.
69. Burger D, Travis S. Conventional Medical Management of Inflammatory Bowel Disease. Gastroenterology 2011;140:1827-1837.
70. Reese GE, Nanidis T, Borysiewicz C, et al. The effect of smoking after surgery for Crohn's disease: a meta-analysis of observational studies. Int J Colorectal Dis 2008;23:1213-1221.
71. Sutherland LR, Ramcharan S, Bryant H, et al. Effect of cigarette smoking on recurrence of Crohn's disease. Gastroenterology 1990;98:1123-1128.
72. Cosnes J, Carbonnel F, Beaugerie L, et al. Effects of cigarette smoking on the long-term course of Crohn's disease. Gastroenterology 1996;110:424-431.
73. Timmer A, Sutherland LR, Martin F. Oral contraceptive use and smoking are risk factors for relapse in Crohn's disease. The Canadian Mesalamine for Remission of Crohn's Disease Study Group. Gastroenterology 1998;114:1143-1150.
74. Cosnes J, Beaugerie L, Carbonnel F, Gendre JP. Smoking cessation and the course of Crohn's disease: an intervention study. Gastroenterology 2001;120:1093-1099.
75. Stein RB, Hanauer SB. Comparative tolerability of treatments for inflammatory bowel disease. Drug Saf 2000;23:429-448.

Chapter 2A

Serious events with infliximab in patients with inflammatory bowel disease: a 9-year cohort study in the Netherlands

Drug Safety 2008; 31(12):1135-1144

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The tumour necrosis factor-α inhibitor infliximab is incorporated in the treatment guidelines for patients with inflammatory bowel disease (IBD). However, concerns about serious adverse events such as infections, malignancies and death do exist. The aim of this study was to evaluate the occurrence of serious events with infliximab during 9 years in a single-centre cohort of patients with IBD. Consecutive patients (>18 years) with a proven diagnosis of IBD who started treatment for IBD with infliximab at our referral centre in the Netherlands, from June 1999 to October 2007, were included. Infusion data were collected prospectively, medical records were reviewed retrospectively. All serious events were recorded and scored in the following categories: events leading to hospitalisation, infections, malignancies and death. Severity and possible relationship to the use of infliximab were assessed for every serious event. 147 patients (33% male, mean age first infusion 38 years, standard deviation = 12) received a total number of 1924 infusions (median per patient = 10, range 1 - 70). A total of 89 patients (61%) were hospitalized during follow-up, involving a total of 300 hospitalizations. Of these, 60 hospitalizations (20%) were considered at least possibly related to the use of infliximab. In 21 hospitalizations, the occurrence of a serious infection was considered at least possibly related to infliximab. Of all hospitalized patients, 70 patients (79%) underwent 139 surgical procedures, of which 70 surgeries (50%) were gastrointestinal related. 9 patients (6%) developed malignancies during follow-up: 4 colorectal carcinomas, 1 carcinoid tumour with another primary signet-ring cell carcinoma of the small bowel, 1 breast cancer, 2 skin cancers and 1 superficial melanoma. During follow-up, 8 patients (5%) died: 6 as a result of malignancies, 1 patient as a result of a complication of short bowel syndrome and 1 patient due to unknown reasons. Patients who developed malignancies tended to have a longer disease duration than those who did not.
Clinicians prescribing biological therapies should be aware of the development of serious events in their patients. Thorough follow-up of all patients during treatment with infliximab is warranted. If infliximab is considered in patients with IBD not responding to conventional treatment, efforts to exclude other possible underlying causes for worsening of symptoms should be made. Careful prescribing and monitoring during follow-up remains necessary.

Introduction

Inflammatory bowel disease (IBD) affects the lives of millions of people in the developed countries. The two major types of inflammatory bowel disease are Crohn's disease (CD) and ulcerative colitis (UC). It is commonly thought that inflammatory bowel disease is caused by an exaggerated immune response to commensal bacteria in genetic susceptible individuals.[1,2] Therefore, targeting this immune response plays an important role in the therapy of inflammatory bowel disease.

The chimeric (partly human, partly murine) monoclonal antibody infliximab (Remicade, Centocor, Malvern, PA) which targets tumour necrosis factor α (TNF-α) is incorporated in the treatment guidelines for patients with inflammatory bowel disease, based on positive results in randomized clinical trials.[3-5] In short, patients who are unable to remain in remission despite adequate treatment with immunosuppressive drugs, patients with intolerance to conventional treatment (i.e. corticosteroids and immunosuppressant's) and patients with enterocutaneous fistulas not responding to conventional therapies, are eligible for treatment with infliximab.[6] Besides its prescription for IBD, infliximab is prescribed in patients with other auto inflammatory diseases like rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis and plaque sporiasis.

Despite the fact that infliximab has benefit in a certain population of IBD patients not responding to conventional therapies, concerns about serious and potentially severe side effects do exist. Side effects of infliximab on the short term are known from clinical trials and cohort studies, such as (acute) infusion reactions and infections.[7] Clinical evidence is now emerging on long term safety and efficacy with infliximab. The use of infliximab is clearly associated with a increased risk of infection. Serious opportunistic infections; mycobacterium infections, pneumocystis jiroveci (carinii) pneumonia, pulmonary actinomycosis and others have been described after the use of infliximab, as a result of suppression of T-cell mediated immunity.[8-11]

Another point of concern in the use of biologicals in inflammatory bowel disease is the development of malignancies, especially lymphomas. In 2006, Bongartz et al. published a meta-analysis on the risk of malignancies in patients with rheumatoid arthritis treated with infliximab in randomised clinical trials.[12] The outcome of this analysis showed a dose-related increased risk for the development of malignancies. Furthermore, the development of hepatosplenic T-cell lymphoma, a very rare subtype of peripheral T-cell non-Hodgkin lymphoma, in paediatric and young adult patients treated with infliximab has been described.[13] Although it is unclear if infliximab plays a role in the pathogenesis of this lymphoma, clinicians prescribing infliximab in paediatric patients have been warned by the manufacturer to pay attention to possible development of this very rare lymphoma in their patients.

In this single centre cohort study we evaluated the occurrence of serious events after the onset of infliximab treatment of patients with IBD, with a follow-up of nine years.

Methods

Patients

Consecutive patients (>18yrs) with a proven diagnosis of IBD who started treatment with infliximab from June 1999 through October 2007 at our centre were included in our study cohort. Our centre, The Department of Gastroenterology and Hepatology of the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, is a IBD referral centre. Referred patients and paediatric patients who had received infliximab elsewhere by the time of first infusion, were excluded. The dose of infliximab administered was based on patient's weight and adjusted to a dose of 5 mg/kg bodyweight, given as a 2-hours intravenous infusion. Indications for treatment with infliximab were based on Dutch treatment algorithms by that time available.[14,15]

Serious events with infliximab

Infusion data and doses were collected prospectively and complete medical records were reviewed retrospectively over the study period. The following variables related to infliximab were recorded: indication for infliximab, dose, duration and co-medication (corticosteroids and immune suppressants) and the treatment strategy (on demand versus scheduled maintenance treatment). At the time we started with administering infliximab, most patients were on an 'on demand' schedule. However, when evidence came available indicating that an on demand strategy was associated with the development of antibodies to infliximab,[16] patients switched to a maintenance strategy. Furthermore, the phenotype according to the Montreal Classification,[17] gender, age at start of infliximab treatment, and prior use of medication within one year before starting administering infliximab for a period of at least three months were abstracted.

Acute infusion reactions were defined as any adverse event occurring during infusion or within a period of two hours after infusion, and delayed infusion reactions as reactions occurring from 24 hours to 14 days after treatment with infliximab, according to Cheifetz *et al.*[18] A serious event was defined as any unfavourable event since the start of infliximab, from one of the following categories: infections, malignancies and death.

Hospitalisations, length of stay and surgical procedures were assessed from medical records and electronic patient records; subdivided into gastrointestinal related and other. Furthermore, events were scored for severity and likelihood of relationship to infliximab; grading of adverse events was performed by adapting the grading of the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CT-CAE, table 2.1).[19] Assessment of causality was performed by using a modified WHO-Uppsala Monitoring Centre (UMC)scale of case causality assessment (table 2.2).[20] Scoring was performed by an experienced gastroenterologist (DJ) blinded for information that could lead to a patient's identity.

All events during follow-up were graded by the NCI CT-CAE, and defined as related to the use of infliximab if causality was scored 3 (possible) or 4 (probable), following the modified WHO-UMC scale of case causality assessment as described in table 2.2.

Table 2.1 Grading system of adverse events adapted from the National Cancer Institute Common Terminology Criteria for Adverse Events[19]

Grade	Description	No. of events	Events related to infliximab use [n (%)][a]
0	No adverse event (absent) or within normal limits	-	-
1	Mild adverse event (minor; no specific medical intervention; asymptomatic laboratory findings only; radiographic findings only; marginal clinical relevance)	1	0 (0)
2	Moderate adverse event (minimal intervention; local intervention; noninvasive intervention)	73	18 (25)
3	Severe and undesirable adverse event (significant symptoms requiring hospitalization or invasive intervention; transfusion; elective interventional radiological procedure; therapeutic endoscopy or operation)	216	46 (21)
4	Life-threatening or disabeling adverse event (complicated by acute, life-threatening metabolic or cardiovascular complications such as circulatory failure, haemorrhage, sepsis. Life-threatening physiological consequences; need for intensive care or emergent invasive procedure; emergent interventional radiological procedure, therapeutic endoscopy or operation)	23	18 (78)
5	Death related to adverse event	7	6 (86)

[a]Events are defined related to the use of infliximab if causality was scored 3 (possible) or 4 (probable), following the modified WHO Uppsala Monitoring Centre scale of case causality assessment as described in table 2.2 NA = not applicable.

Table 2.2 Modified WHO-Uppsala Monitoring Centre scale of case causality assessment.[20]

Grade	Description
1	Unrelated: a causal relationship can be definitively excluded and another documented cause of the event is most plausible
2	Unlikely: a causal relationship is improbable and another documented cause of the adverse event is most plausible
3	Possible: a causal relationship is clinically/biologically plausible and there is a plausible time sequence between onset of the adverse event and administration of infliximab
4	Probable: a causal relationship is clinically/biologically highly plausible and there is a plausible time sequence between onset of the adverse event and administration of infliximab and there is a reasonable response on withdrawal

Statistics

Frequency tables were compiled describing characteristics of the included patients at the time of their first infusion with infliximab. The number of hospitalizations were analysed on a patient level, and defined as related to the use of infliximab if causality was scored 3 or 4. This procedure was repeated for both serious infection and surgery as the reason for hospitalization. All patients who were hospitalized, whether or not the occurrence of a serious infection was the main reason, were compared with regard to the treatment strategy (on demand vs scheduled maintenance) and the use of co-medication, which was defined as use over a period of at least 6 months. Patients who developed malignancies during follow-up were compared with the remaining patients for disease severity (defined as presence of fistulas), duration of disease, cumulative dose of infliximab and the use of concomitant medication using Student's t-test and Pearson's chi-squared test. The annual mortality and infection rate was calculated by dividing the number of deceased patients or patients with infection by the duration of follow-up in years. All calculations were performed using SAS software (version 8.2; SAS Institute Inc., Cary, NC, USA). All p-values calculated were two-tailed and the alpha level of significance was set at 0.05.

Results

Patients

A total of 147 patients (33% male) were included; baseline characteristics at time of first infusion with infliximab are given in table 2.3. At baseline, mean age was 38 years (standard deviation = 12) with a mean disease duration of 16 years. Primary indication for treatment was luminal CD in 55 patients (37%), fistulizing disease in 80 patients (54%), UC in 9 patients (6%) and indeterminate colitis in 3 patients (2%). Involvement of different parts of the intestinal tract was scored using the Montreal classification: in patients with CD it was limited to the small bowel in 26 patients (18%), ileocolonic in 63 patients (43%) and colonic in 42 patients (29%). A total number of 1924 infusions were given with a median per patient of 10 (range 1–70), over a median follow-up of 59 months (range 1–99). Patients were followed for a total of 674 patient years. Twenty-eight patients (20%) received infliximab for less than 6 months.

Infusion reactions

A total number of 16 acute infusion reactions were seen in 12 patients (8%). When patients developed acute infusion reactions, infusion was discontinued and patients received intravenous corticosteroids and a selective histamine H1 antagonist (clemastine). Based on the clinical response to these agents, infliximab was re-administered at a slower infusion rate and discontinued if symptoms reoccurred. The majority of acute infusion reactions occurred during the fifth and sixth infusions and most patients who developed acute infusion reactions were receiving scheduled maintenance therapy (figure 2.1). There were 12 delayed infusion reactions, occurring in 10 patients (7%).

Table 2.3 Baseline characteristics of 147 patients with inflammatory bowel disease at start of infliximab treatment

Characteristic	No of patients
Mean age, years (SD)	38 (±12)
Male gender (%)	49 (33)
Mean disease duration, years (range)	16 (0-41)
Indication for infliximab treatment (%)	
luminal CD	55 (37)
fistulizing CD	80 (54)
UC	9 (6)
indeterminate colitis	3 (2)
Montreal classification CD (%)	
ileum	26 (18)
colon	42 (29)
ileocolonic	63 (43)
ileum + isolated upper disease	1 (1)
colon + isolated upper disease	1 (1)
ileocolonic + isolated upper disease	4 (3)
only perianal disease	1 (1)
Montreal classification UC (%)	
ulcerative proctitis	1 (1)
left-sided	3 (2)
pancolitis (extensive colitis)	5 (3)
Prior medication[a]	
5-aminosalicylic acid	66 (45)
corticosteroids	113 (77)
antibacterials	15 (10)
immunosuppressants	90 (61)
Concomitant medication at first infusion (%)	
corticosteroids	102 (69)
immunosuppressants	93 (63)

[a]Medication had to be used over a period of at least 3 months during the previous year. CD = Crohn's disease; UC = ulcerative colitis.

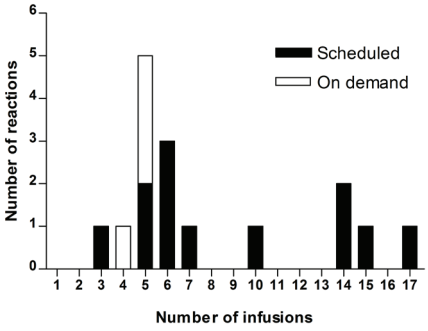

Figure 2.1 Acute infusion reactions to infliximab. Patients were stratified according to their treatment regimen (on demand vs scheduled).

The overall median number of hospitalizations per patient was 2, ranging from 1-19. Of the hospitalizations at least possible related to infliximab, the median number per patient was 1 (range 1-6). Overall median length of stay was 7 days (range 1-70) and in the group with hospitalization at least possibly related to infliximab, it was 8 days (range 1-48). In 57 hospitalizations (involving 36 patients), the occurrence of a serious infection was the main reason for hospitalization; in 21 patients (24%) this was considered at least possibly related to infliximab. The following infections were recorded: abscess (58%), gastroenteritis (16%), urinary tract infection (11%), pneumonia (5%), sepsis (9%) and not categorizable (5%). It should be noticed that some patients had multiple types of infection. The annual infection rate was 5%. Of all 89 hospitalized patients, 70 (79%) underwent 139 surgical procedures of which 70 (50%) were gastrointestinal related. Most patients were hospitalised because of their disease. No significant differences were found regarding different treatment schedules (on demand vs scheduled) and between the different groups of co-medication, i.e. corticosteroids, immunosuppressant's or both (table 2.4).

Malignancies

During follow-up, nine patients (6%) developed malignancies (table 2.5). Four colorectal carcinomas, one carcinoid tumour with another primary signet-ring cell carcinoma of the small intestine, one breast cancer, one basal cell carcinoma, one squamous cell carcinoma of the skin and one superficial melanoma were seen. All malignancies except one were judged at least possibly related to the use of infliximab (table 2.5). The relationship is unlikely in the case of a 56-year-old man with a 14-year history of CD, who was hospitalized because of general deterioration, dyspnoea, diarrhoea and fatigue. Besides his CD, he had a history of ankylosing spondylitis and a Dukes BII colon tumour, which had been completely removed 14 years earlier. During his hospitalization he received three infusions with infliximab. Six days after being discharged, he was re-admitted to the hospital because of a massive gastrointestinal bleeding and died during an emergency operation.

At autopsy, an adenocarcinoma was seen in the ileorectal region, with invasion of the iliac vessels. Three patients continued infliximab after the diagnosis of malignancy. Besides patients with basal cell carcinoma and superficial melanoma, there was one patient with metastatic breast cancer. This 52-year-old woman had a history of severe fistulizing perianal CD for 26 years and was responding well to infliximab. When diagnosed with breast cancer, her treating physicians strongly advised her to stop further treatment. Despite that, she decided to continue treatment with infliximab with the argument that she would rather have a few good months with limited CD activity and a potentially reduced life expectancy, than having a few more months with both active perianal CD and breast cancer.

Patients who developed malignancies were compared with patients who did not develop malignancies; no significant differences were found in age of onset, concomitant medication and existence of fistulae. Patients who developed malignancies had received a median number of 17 infusions with infliximab, compared with a median number of 10 infusions in patients who did not. The cumulative dose of infliximab received, which has been associated with the development of malignancies, did not show significant differences either; the mean dose in the group with malignancies was 1540 mg (600 - 35000 mg) versus a mean of 2500 mg (300 - 21940 mg). However, a significant difference was seen in duration of disease ($p < 0.01$); patients with malignancies had a mean duration of disease of 29 years (± 7.3 years), compared with a mean duration of disease of 15 years (± 8.6 years), in patients who did not develop malignancies.

Table 2.4 Summary of concomitant medication and treatment strategy regarding hospitalization and infection

Medication	Hospitalizations			Infections		
	no. of events (n = 300)	no. of patients (n = 89)	median no. of events per patient	no. of events (n = 57)	no. of patients (n = 36)	median no. of events per patient
No concomitant medication	6	3	2	4	3	1
Corticosteroids	33	10	3	5	4	1
Immunosuppressants	57	16	2	7	4	1
Combination	136	31	3	28	14	1
Unknown	2	1	2	1	1	1
<6 Mo of infliximab	66	28	2	12	10	1
Infliximab						
on demand	177	44	2	38	20	1
scheduled	123	45	2	19	16	1

Table 2.5 Characteristics of patients who developed malignancies

Sex/ Age (y)	Disease	Disease Duration (y)	No. of infusions	Cumulative dose received (mg)	Time since last infusion	Concomitant medication	Type of malignancy	Follow-up	Likelihood score[a]
M/56	CD[b]	31	4	1600	32 mo	AZA	CRC	Patient died 5 mo after diagnosis	3 (Possible)
F/48	CD[b]	32	4	1200	7 wk	GCS, 5-ASA	CRC	Patient underwent surgery and received radiation therapy and died 19 mo after diagnosis	3 (Possible)
M/63	CD[b]	37	3	900	26 mo	Mesalazine	CRC	Patient died nearly 6 mo after diagnosis	3 (Possible)
F/44	CD[b]	21	9	3400	6 wk	GCS, AZA, 5-ASA	Basal cell carcinomas	Basal cell carcinomas could be removed completely and infliximab was readministered	3 (Possible)
F/52	CD[b]	31	47	23500	2 wk	5-ASA, AZA (later followed by MTX)	Melanoma	Melanoma could be removed completely and infliximab was continued	4 (Probable)
M/56	CD	14	3	900	6 d	GCS, AZA	CRC	Patient died during an emergency operation because of massive bleeding caused by a tumour in the ileorectal region that had grown into the iliac vessels	2 (Unlikely)
F/45	CD	18	29	11600	2 mo	5-ASA, AZA	Carcinoid tumour,[c]	Patient received chemotherapy and died nearly 7 mo after diagnosis	3 (Possible)
F/52	CD[b]	26	12	4348	2 mo	GCS, 5-ASA,[d] 3 mo of MTX	Breast cancer	Patient underwent surgery and received another ten infusions with infliximab and died 28 mo after diagnosis	3 (Possible)
F/52	CD	18	2	600	17 mo	AZA	Squamous cell carcinoma	Squamous cell carcinoma could be removed completely	3 (Possible)

[a] Likelihood of malignancy being related to the use of infliximab (Modified WHO-UMC scale as described in table 2.2). [b] CD patients with fistula. [c] This patient developed a carcinoid tumour with another primary signet-ring cell carcinoma (linitis plastica type) and lymphangitis carcinomatosa. [d] Only during 1st year of treatment. AZA = azathioprine; 5-ASA = 5-aminosalicylic acid; CD = Crohn's disease; CRC = colorectal cancer F = female; M = male; MTX = methotrexate.

Deaths

During follow-up, eight patients (5%) died: six as a result of malignancies (all patients with colorectal carcinomas, breast cancer or primary signet-ring cell carcinoma of the small intestine), one patient as a result of a complication of short bowel syndrome and for one patient the reason was unknown. The annual mortality was 1.2%.

Discussion

In our 9 years of single-centre experience a total of 147 patients with IBD received nearly 2000 infusions with infliximab. In 12 patients (8%) an acute infusion reaction was seen and 10 patients (7%) developed delayed infusion reactions. Our rate of acute infusion reactions on a patient level is comparable with reported rates in other studies.[18] With regard to the development of acute infusion reactions, most treatment algorithms state that vital signs should be monitored during infusion.[18,21] We recently showed that scheduled monitoring of vital signs during infusion neither indicated nor predicted development of acute infusion reactions.[22] Sixty - one percent of all patients were hospitalized after the start of infliximab treatment during follow-up. In 14% of all patients, the main reason for hospitalization was an infection that was considered at least possibly related to the use of infliximab. Nine patients developed malignancies and subgroup analysis, comparing patients with and without malignancies, showed a significant difference in duration of disease. During follow-up, eight patients died, the majority as a result of malignancies. In large, controlled clinical trials of maintenance therapy with infliximab in patients with CD, the annual rate of serious infections with infliximab ranged from 4% to 4.6%.[3,23] Our annual infection rate is comparable to these studies, but slightly higher than demonstrated by follow-up data from clinical practice, which showed an annual incidence of serious infections of 1.2 - 2.1%.[24,25] The TREAT (The Crohn's Therapy, Resource, Evaluation and Assessment Tool) registry, a large scale, ongoing, observational registry of patients treated for CD, showed no significant differences in serious infection between patients treated with infliximab and patients not treated with infliximab in the latest published evaluation.[26] However, a recent case-control study showed that immunosuppressive medication such as corticosteroids, azathioprine/ mercaptopurine and infliximab, especially when used in combination, is associated with an increased risk of opportunistic infections in patients with IBD.[27]

In our cohort, only those infections for which hospitalization was required were registered. As a consequence, we underestimated the true rate of infections in patients treated with infliximab. Furthermore, patients could be treated for less severe infections by their general practitioner. Since we reviewed the medical charts retrospectively, this information could not be reliably retrieved.

Concerns do exist about the development of lymphomas in patients treated with infliximab. In our cohort, only solid tumours were found with the majority being colorectal cancer. Patients with IBD, especially patients with UC and to a lesser degree patients with CD, are at greater risk of developing colorectal cancer, depending on risk factors such as duration and severity of disease and simultaneous primary

sclerosing cholangitis.[28] In our subgroup analysis comparing patients with and without malignancies (patients with malignancies being the group who contributed most to the number of patients who died during follow-up), only duration of disease was significantly different. Duration of colitis is a significant contributor in the development of colorectal cancer in patients with IBD.[28] Besides patients with UC, patients with CD have an increased risk of developing colorectal cancer.[29,30] A general consensus exists that the same contributing factors for colorectal cancer in patients with UC apply for patients with CD.[30] In our cohort, a causal relationship between the use of infliximab and the development of solid tumours can not be made; the malignancies seem to be more related to underlying disease than to the use of infliximab. However, these malignancies developed at a relative young age. This indicates that careful monitoring of patients with IBD for signs which could be attributable to a malignancy, is necessary to detect serious events at an early stage.

One patient was treated with infliximab for worsening of gastrointestinal symptoms, which on retrospective investigation were found to be caused by colorectal cancer. Since IBD-related symptoms may have other causes such as malignancy, clinicians should consider a diagnostic work-up before starting treatment with infliximab. We retrospectively checked the medical records of the four patients who developed colorectal cancer with regard to endoscopies performed. None of the colorectal cancers was discovered during a previous colonoscopy or sigmoidoscopy. Three of four patients underwent at least two colonoscopies in the 3-year period before the colorectal cancer was diagnosed.

Another patient, who developed breast cancer, insisted on continuing treatment with infliximab. This is in line with a recent study by Johnson *et al.*, which revealed that patients with CD are willing to accept an elevated risk of serious adverse events in exchange for clinical efficacy with regard to their disease.[31]

Our annual mortality rate of 1.2% is in accordance with previous double-blind clinical trials with infliximab and follow-up data from clinical trials, reporting incidences ranging from 0.7-1.3%[3,23] and from 1.2 - 1.3%,[24,25] respectively. However, as pointed out by Lichtenstein *et al.*,[26] this mortality rate is in accordance with published mortality rates in historical cohort studies before infliximab was introduced, with annual mortality rates of 1.3% in patients with CD.[32,33] In the multivariate regression analysis of the TREAT registry, only the use of prednisone was an independent predictor of both serious infection and death.[26] Our study was underpowered to perform such an extensive multivariate analysis.

The use of infliximab in fistulizing CD is associated with a reduced number and length of hospitalizations.[34] In our cohort, a considerable number of patients were hospitalised (61%) and 70 gastrointestinal surgeries were performed during follow-up. In our centre, infliximab given in a conventional 'step-up' approach was administered relatively late in the course of disease. This possibly had consequences for the biological behaviour of the disease, with progression of stricturing and fistula formation. It seems that this approach is not able to substantially change the course of disease and surgery remained inevitable in a large number of patients.

One limitation of this study is the lack of a comparator, for example, a group of patients with similar disease severity, medication use, duration of disease and number of bowel segments involved but not treated with infliximab. As a consequence we can not report 'higher rates', but only high rates. Furthermore, no risk ratios could be calculated. Other factors such as concomitant use of corticosteroids and immune suppressants could likely contribute to the reported event rate here. Therefore, the reported adverse effects might be caused by conventional medication used together with infliximab. As conventional medication causes serious events as well,[35] it is difficult to differentiate. Another limitation with regard to this retrospective study is that we were not able to differentiate between patients who fully responded to infliximab therapy and those who responded only partially or in whom response was lost, since response to infliximab was not measured prospectively following a standard protocol and definitions.
While long term data on other TNF-α blocking agents used in the treatment of inflammatory bowel disease (e.g. adalimumab) are lacking, infliximab is the only TNF-α inhibitor suitable for long term safety analysis at this moment. Therefore, no comparison in long-term safety can be made. Although not studied in head-to-head trials, we would stress precautions concerning other anti TNF-α agents used in the treatment of inflammatory bowel disease as well.

Conclusion

Although infliximab is of great value in the treatment of IBD, clinicians prescribing biological therapies should be aware of the development of serious events in their patients. There might be an indication for surveillance screening for colorectal cancer in this subset of patients who are under infliximab treatment. Thorough follow-up of these patients during treatment is warranted. If infliximab is considered in patients with IBD not responding to conventional treatment, efforts to exclude other possible underlying causes for worsening of symptoms should be made.

References

1. Sartor RB. Microbial influences in inflammatory bowel diseases. Gastroenterology 2008;134:577-594.
2. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448:427-434.
3. Hanauer SB, Feagan BG, Lichtenstein GR, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet 2002;359:1541-1549.
4. Rutgeerts P, D'Haens G, Targan S, et al. Efficacy and safety of retreatment with anti-tumor necrosis factor antibody (infliximab) to maintain remission in Crohn's disease. Gastroenterology 1999;117:761-769.
5. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med 2005;353:2462-2476.
6. Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. Lancet 2007;369:1641-1657.
7. Hanauer SB. Review article: safety of infliximab in clinical trials. Aliment Pharmacol Ther 1999;13:16-22.
8. Keane J. TNF-blocking agents and tuberculosis: new drugs illuminate an old topic. Rheumatology 2005;44:714-720.
9. Salvana EMT, Cooper GS, Salata RA. Mycobacterium other than tuberculosis (MOTT) infection: An emerging disease in infliximab-treated patients. J Infect 2007;55:484-487.
10. Kaur N, Mahl TC. Pneumocystis jiroveci (carinii) pneumonia after infliximab therapy: A review of 84 cases. Dig Dis Sci 2007;52:1481-1484.
11. Cohen RD, Bowie WR, Enns R, et al. Pulmonary actinomycosis complicating infliximab therapy for Crohn's disease. Thorax 2007;62:1013-1014.
12. Bongartz T, Sutton AJ, Sweeting MJ, et al. Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies - Systematic review and meta-analysis of rare harmful effects in randomized controlled trials. JAMA 2006;295:2275-2285.
13. Mackey AC, Green L, Liang LC, et al. Hepatosplenic T cell lymphoma associated with infliximab use in young patients treated for inflammatory bowel disease. J Pediatr Gastroenterol Nutr 2007;44:265-267.
14. van Berge Henegouwen GP. Consensus for infliximab treatment of patients with Crohn's disease. Ned Tijdschr Geneeskd 2000;144:1844-1845.
15. Hommes DW, Oldenburg B, van Bodegraven AA, et al. Guidelines for treatment with infliximab for Crohn's disease. Neth J Med 2006;64:219-229.
16. Baert F, Noman M, Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med 2003;348:601-608
17. Satsangi J, Silverberg MS, Vermeire S, et al. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut 2006;55:749-753.
18. Cheifetz A, Smedley M, Martin S, et al. The incidence and management of infusion reactions to infliximab: A large center experience. Am J Gastroenterol 2003;98:1315-1324.
19. National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE). Available from http://ctep.cancer.gov/forms/CTCAEv3.pdf [Accessed Oct 2008]
20. WHO-Uppsala Monitoring Centre (UMC) scale of case causality assessment. Available from http://www.whoumc.org/graphics/4409.pdf [Accessed Oct 2008]
21. Sandborn WJ, Hanauer SB. Infliximab in the treatment of Crohn's disease: A user's guide for clinicians. Am J Gastroenterol 2002;97:2962-2972.

22. de Vries HS, van Oijen MG, van Hoven-van Loo KE, et al. Monitoring vital signs during infusion with infliximab does neither indicate nor predict development of acute infusion reactions. J Clin Gastroenterol 2009;43:387-388.
23. Sands BE, Anderson FH, Bernstein CN, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. N Engl J Med 2004;350:876-885.
24. Ljung T, Karlen P, Schmidt D, et al. Infliximab in inflammatory bowel disease: clinical outcome in a population based cohort from Stockholm County. Gut 2004;53:849-853.
25. Colombel JF, Loftus EV, Tremaine WJ, et al. The safety profile of infliximab in patients with Crohn's disease: The Mayo Clinic experience in 500 patients. Gastroenterology 2004;126:19-31.
26. Lichtenstein GR, Feagan BG, Cohen RD, et al. Serious infections and mortality in association with therapies for Crohn's disease: TREAT registry. Clin Gastroenterol Hepatol 2006;4:621-630.
27. Toruner M, Loftus EV, Harmsen WS, et al. Risk factors for opportunistic infections in patients with inflammatory bowel disease. Gastroenterology 2008;134(4):929-36.
28. Xie JL, Itzkowitz SH. Cancer in inflammatory bowel disease. World J Gastroenterol 2008;14:378-389.
29. Lutgens MW, van der Heijden GJ, Vleggaar FP, et al. A comprehensive meta-analysis of the risk of colorectal cancer in ulcerative colitis and Crohn's disease. Gastroenterology 2008;134:A33-A34.
30. Zisman TL, Rubin DT. Colorectal cancer and dysplasia in inflammatory bowel disease. World J Gastroenterol 2008;14:2662-2669.
31. Johnson FR, Ozdemir S, Mansfield C, et al. Crohn's disease patients' risk-benefit preferences: Serious adverse event risks versus treatment efficacy. Gastroenterology 2007;133:769-779.
32. Jess T, Winther KV, Munkholm P, et al. Mortality and causes of death in Crohn's disease: Follow-up of a population-based cohort in Copenhagen County, Denmark. Gastroenterology 2002;122:1808-1814.
33. Loftus EV, Silverstein MD, Sandborn WJ, et al. Crohn's disease in Olmsted County, Minnesota, 1940-1993: Incidence, prevalence, and survival. Gastroenterology 1998;114:1161-1168.
34. Lichtenstein GR, Yan SK, Bala M, et al. Infliximab maintenance treatment reduces hospitalizations, surgeries, and procedures in fistulizing Crohn's disease. Gastroenterology 2005;128:862-869.
35. Stein RB, Hanauer SB. Comparative tolerability of treatments for inflammatory bowel disease. Drug Saf 2000;23:429-448.

Chapter 2B

Safety of infliximab in inflammatory bowel disease needs to be debated

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To the Editor,

With great interest we read the article by Caspersen *et al.* in which they investigated the long-term safety of infliximab in patients with inflammatory bowel disease (IBD) in a national Danish population-based cohort.[1] They scored adverse events in up to 47% of all patients and concluded that infliximab was generally well tolerated. Their conclusion based on these numbers is debatable, and is also based on a retrospective study, with subsequent non-standardized underreporting of adverse events as compared with prospective clinical trials. Moreover, the data of the Danish IBD cohort was gathered over a relatively short follow-up period.
In table 2.6, we compare their study with 3 other recently published retrospective cohort studies about the long-term safety of infliximab in patients with IBD from Belgium, Scotland, and The Netherlands.[2-4]

Table 2.6 Characteristics of the Four Recently Reported Studies on the Long-Term Safety of Infliximab

	Caspersen *et al.*[1]	Fidder *et al.*[2]	Lees *et al.*[3]	de Vries *et al.*[4]
Number of treated patients	651	734	202	147
Median age of starting therapy, years	31.6 (range, 9.7-92.4)	35 (IQR, 25-35)	30.8 (IQR, 22.0-42.9)	38 (SD, 12)
Median disease duration before first infusion, years	5 (range, 0-38)	7 (IQR, 2-15)	4.9 (IQR, 1.2-11.0)	16 (range, 0-41)
Number of infusions	3351	7276	718	1941
Median number of infusions per patient	3	6	3 for CD, 1 for UC	10
Follow-up period (patient-years)	-	3775	620	674
Median follow-up period, months	29.1	58	28.8	59
Number of infections[a]	69 (63 patients)	59 (48 patients)	60 (42 patients)	57 (36 patients)
Annual infection rate, %	-	1.3%	7%	5%
Number of malignancies, %	4 (0.6)	21 (2.8)	6 (3.0)	9[b] (6.1)
Mortality, %	13 (2.0)	12 (1.6)	7 (3.5)	8 (5.4)
Annual Mortality rate, %	-	0.3	1.1	1.2

IQR, interquartile range; CD, Crohn's disease; UC, ulcerative colitis.
[a]In one of the studies (de Vries *et al*), only those patients who underwent hospitalization for their infections were included. In others, hospitalization was not required in case of an infection. Therefore, the given numbers are not completely comparable since inclusion criteria are different between these studies.
[b]There were 9 malignancies in 8 patients.

The Denmark IBD population treated with infliximab developed fewer malignancies (4; 0.6%) compared with the cohorts from Belgium (21; 2.8%), Scotland (6; 3.0%), and The Netherlands (9; 6.1%). Most likely this could be explained by a relatively short follow-up period (median follow-up period, 29.1 months) compared with the cohorts from Belgium and The Netherlands (median follow-up periods, 58 and 59 months, respectively), and therefore underestimation of the true rate of malignancies. The raised concern of developing malignancies in patients treated with anti–tumor necrosis factor, in combination with the fact that carcinogenesis is a process requiring many years of exposure to disease or treatment, makes it necessary to have a long term follow-up period to detect malignancies. This is emphasized further with the significant association with duration of disease and the development of malignancies in the studies from Belgium and The Netherlands. This indicates that thorough follow-up evaluation of patients with IBD for the development of malignancies is warranted. The question remains whether the high rate of malignancies is related to disease, treatment, or a synergistic effect. The Belgian cohort did include a control group of IBD patients treated in the same center and period. They compared IBD patients treated with infliximab with patients not receiving any biological therapy and found no differences in terms of mortality, infection rates, and malignancies.[2] These findings need to be reproduced in other centers, but give us some first impressions regarding causality with IBD.

References

1. Caspersen S, Elkjaer M, Riis L, et al. Infliximab for inflammatory bowel disease in Denmark 1999-2005: clinical outcome and follow-up evaluation of malignancy and mortality. Clin Gastroenterol Hepatol. 2008;6:1212-1217.
2. Fidder HH, Schnitzler F, Ferrante M, et al. Long-Term Safety of Infliximab for the treatment of Inflammatory Bowel Disease: A Single Center Cohort Study. Gut. 2009;58:501-508.
3. Lees CW, Ali A, Thompson AI, et al. The safety profile of anti-TNF therapy in inflammatory bowel disease in clinical practice: analysis of 620 patient-years follow-up. Aliment Pharmacol Ther. 2009;29:286-297.
4. de Vries HS, van Oijen MG, de Jong DJ. Serious events with infliximab in patients with inflammatory bowel disease: a 9-year cohort study in the Netherlands. Drug Saf. 2008;31:1135-1144.

Chapter 2C

Monitoring vital signs during infusion with infliximab does neither indicate nor predict development of acute infusion reactions

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Most treatment algorithms involving infliximab state that before and during infusion, vital signs [for example, mean arterial pressure (MAP) and pulse] should be monitored in case acute infusion reactions develop.[1–3] Although easy to perform, monitoring and registering vital signs is time consuming and has consequences for the management of infusion units. In this era of increasing healthcare costs, nursing services should be appropriate to be cost effective.[4] In the present study, we questioned the value of monitoring vital signs before and during infusion with infliximab on clinical relevant outcomes, that is, the development of acute infusion reactions. Consecutive patients with a proven diagnosis of inflammatory bowel disease (IBD) starting infliximab in the period June 1999 till October 2007 in the Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands, a referral center for IBD, were included. Referred patients from other clinics who had received infliximab outside our clinic and pediatric patients (age < 18 years), by the time of first infusion, were excluded. The dose of infliximab administered was based on patient's weight and adjusted to a dose of 5 mg/ kg bodyweight. Indications for treatment with infliximab were based on Dutch treatment algorithms available at that time.[5,6]

Vital signs (temperature, pulse, and blood pressure) were prospectively monitored before (baseline) and during all infusions on a structured datasheet and combined with clinical data on infusion reactions from medical files. We calculated mean arterial pressure (MAP) as follows:

$$MAP = \frac{1}{3} \times (2 \times diastolic\ blood\ pressure + systolic\ blood\ pressure)$$

Temperature was monitored by using a tympanic thermometer (Genius First Temp Model 3000A, Sherwood Medical, Sussex, United Kingdom). During an acute infusion reaction, time of onset, blood pressure, and pulse were measured and recorded on the same datasheet. Pearson's w2 tests were used to find differences between patients with and without the experience of an acute infusion reaction for categorical variables, Fisher exact test was used where appropriate. Student t tests were used to analyze differences in continuous baseline characteristics. To adjust for multitesting, a 2-sided P value below 0.01 was regarded statistical significant. A total number of 151 patients (36% males) were included and received 1941 infusions. The median number of infusions per patient was 10 (range: 1 to 70). The overall incidence of acute infusion reactions to infliximab was 0.82% (17 of 1941 infusions), affecting 13 patients (7.9%). Most documented symptoms were: dyspnea (41%), chest pain (41%), dizziness (35%), and flushing (18%).

After developing an acute infusion reaction, 8 patients (62%) continued treatment and received consecutive infusions with IV corticosteroid and antihistamine prophylaxis. Of these, 3 patients (38%) experienced another acute infusion reaction. Baseline vital signs and body temperature between groups with and without acute infusion reactions were compared: MAP at baseline of the group with an acute infusion reaction (mean 88mm Hg±14) and pulse (mean 78 beats/min±9) compared with MAP (mean 88mm Hg±13) and pulse (mean 76 beats/min±13) in the group

without an acute infusion reaction, showed no significant difference. Temperature at baseline in the group with an acute infusion reaction compared with the group without an acute infusion reaction did not show a significant difference (mean 36.9 °C ± 0.47 compared with mean 36.8 °C ± 0.51). Subgroup analysis in 13 patients who experienced an acute infusion reaction showed no significant difference: MAP at baseline (mean 88 mm Hg ± 14) and pulse (mean 78 beats/ min ± 9) in infusions with an acute infusion reaction, and MAP (mean 83 mm Hg ± 14) and pulse (mean 74 beats/ min ± 11) in infusions without an acute infusion reaction were measured, respectively. During an acute infusion reaction vital signs (mean MAP 90 mm Hg ± 13 and mean pulse 77 beats/ min ± 9) did not show a significant change compared with the baseline of the group who experienced an acute infusion reaction (P = 0.7 and 0.9, respectively).

In conclusion, scheduled monitoring of vital signs during infliximab infusion did neither indicate nor predict development of acute infusion reactions in IBD patients and monitoring of vital signs during an acute infusion reaction did not show significant changes to baseline. Therefore, scheduled monitoring of vital signs during infliximab infusions is not indicated in IBD patients. Our data are not a justification for more distant supervision of infusions, but indicate that infusion reactions are rather recognizable by clinical symptoms than by vital signs. This may lead to a more efficient allocation of nursing resources and will likely decrease healthcare costs and increase patient privacy and satisfaction.

References

1. Panaccione R, Fedorak RN, Aumais G, et al. Canadian Association of Gastroenterology Clinical Practice Guidelines: the use of infliximab in Crohn's disease. Can J Gastroenterol. 2004;18:503-508.
2. Cheifetz A, Smedley M, Martin S, et al. The incidence and management of infusion reactions to infliximab: a large center experience. Am J Gastroenterol. 2003;98:1315-1324.
3. Sandborn WJ, Hanauer SB. Infliximab in the treatment of Crohn's disease: a user's guide for clinicians. Am J Gastroenterol. 2002;97:2962-2972.
4. Potti A, Panwalkar A, Hebert B, et al. Ineffectiveness of measuring routine vital signs in adult inpatients with deep venous thrombosis. Clin Appl Thromb Hemost. 2003;9:163–166.
5. van Berge Henegouwen GP. Consensus for infliximab treatment of patients with Crohn's disease. Ned Tijdschr Geneeskd. 2000;144:1844-1845.
6. Hommes DW, Oldenburg B, van Bodegraven AA, et al. Guidelines for treatment with infliximab for Crohn's disease. Neth J Med. 2006;64:219-229.

Chapter 3

Appropriate infliximab infusion dosage and monitoring: results of a panel meeting of rheumatologists, dermatologists and gastroenterologists

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Infliximab, an anti-TNF biologic agent, is currently indicated and reimbursed for rheumatoid arthritis, ankylosing spondylitis, Crohn's disease (both adult and paediatric), ulcerative colitis, psoriatic arthritis and plaque psoriasis. Development of national and international guidelines for rheumatology, gastroenterology and dermatology, was mostly based on clinical studies and expert opinion. The aim of this study was to compare available guidelines and local protocols for rheumatology, dermatology and gastroenterology, regarding dosage of infliximab, synergy of infliximab with concomitant medication and monitoring of vital signs during infliximab administration, for achieving optimal care. Current international, national and local guidelines on the use of infliximab were reviewed and compared, differences and shortcomings were identified, and optimal treatment schedules discussed during a meeting (July 2008) of clinical experts and researchers from three departments of a Dutch university hospital. Recommended dosages of infliximab are not equal for different indications. Loss of response to infliximab is a common problem encountered within the three medical specialties, but indications for adjustments in treatment schedules are lacking in all of the guidelines. Monitoring of vital signs (blood pressure, pulse, temperature) during infusion with infliximab is common practice and recommended by some guidelines. Routine measurement of vital signs is not of any value in predicting or recognizing acute infusion reactions, in our experience, and this is confirmed by literature on inflammatory bowel disease. Different indications encompass different dosing schedules. National and internal guidelines do not provide advice regarding loss of response. Routine measurement of vital signs during infusion is not valuable in detecting acute infusion reactions and should only be performed in case of an acute infusion reaction. These topics need to be studied in future studies and covered in future guidelines.

Introduction

Rheumatoid arthritis, Crohn's disease, ulcerative colitis, psoriatic arthritis, ankylosing spondylitis and psoriasis are chronic inflammatory diseases. Although the exact causes of these diseases remain unknown, over the past two decades major advances have been made in understanding the inflammatory processes. It is likely that in each of these diseases the innate and adaptive immune system are activated, with subsequent production of proinflammatory cytokines, like tumour necrosis factor alpha (TNF-α).[1-3] Antibodies against TNF-α have been developed for the treatment of several chronic inflammatory diseases, including the monoclonal antibodies infliximab and adalimumab. Infliximab, a chimeric (partly human, partly murine) monoclonal antibody (biological), is the only intravenously administered anti-TNF antibody indicated and reimbursed for all of the following diseases: rheumatoid arthritis, ankylosing spondylitis, Crohn's disease (both adult and paediatric), ulcerative colitis, psoriatic arthritis and plaque psoriasis.

National and international guidelines and consensus statements on the use of infliximab have been developed for each of the three medical specialties involved in treatment with infliximab (i.e. gastroenterology, rheumatology and dermatology) and reflect current use in clinical practice.

In many centres like ours, the care for patients receiving infliximab is combined for patients with autoinflammatory disorders. This emphasizes the need for a combination of guidelines for the treatment with infliximab for patients with these disorders within the involved medical specialities.

Methods

This paper is the product of an expert panel meeting, held by the authors in July 2008. The purposes of this meeting were as follows:

- To identify similarities and differences within international, national and local guidelines and additional consensus statements from the medical specialties currently using infliximab as anti-TNF therapy, with regards to:
    - Indications for infliximab
    - Dosage for initial and maintenance therapy
    - Monitoring of vital signs during infusion with infliximab
    - Synergistic effects with concomitant medication use
- To discuss the following topics: optimal dosage of infliximab, monitoring of vital signs and use of concomitant medication.
- To discuss the optimal strategy in patients who have lost response to infliximab.

Members of the panel were selected, based on each member's clinical and/or research experience with use of infliximab, from the departments of rheumatology, gastroenterology and dermatology from our university hospital. Members from each medical field performed a literature search in their own discipline by searching the MEDLINE database until July 2008, using the keyword 'infliximab', limiting their search to practical guidelines and consensus statements.

Additionally, the National Guideline Clearinghouse, a public resource for evidence-based clinical practice guidelines of the Agency for Healthcare Research and Quality in the United States (http://www.guideline.gov) was searched on guidelines related to infliximab. In addition (local) Dutch guidelines from the medical specialties not accessible by MEDLINE but used in clinical practice were reviewed (for an overview of the reviewed guidelines and consensus statements see table 3.1). Regarding these guidelines and consensus statements, we limited ourselves to the previously identified topics, namely indication, dosage, monitoring, synergy and loss of response (i.e. secondary inefficacy). Results were presented and discussed during the panel meeting. Additionally, hiatuses within guidelines and consensus statements were discussed.

Results

Indication

Infliximab was first approved for patients with Crohn's disease in 1998. Approval for other indications followed in the subsequent years (figure 3.1). In general, patients not responding to conventional therapy and having a moderate to high level of disease activity are eligible for treatment with a biological like infliximab.

Gastroenterology

Crohn's disease patients with extra-intestinal manifestations and fistulizing disease are especially eligible for treatment with infliximab.[4,5] Both the international consensus statements of the American Gastroenterological Association (AGA) and the European Crohn's and Colitis Organisation (ECCO) as well as national guidelines agree that treatment with infliximab is appropriate for patients with inflammatory bowel disease experiencing corticosteroid dependency, glucocorticoid and/or immunomodulative treatment refractoriness or active fistula associated with Crohn's disease.[4, 6–8]

Rheumatology

In rheumatoid arthritis, the international consensus statement on biologicals for the treatment of rheumatoid arthritis, which is updated nearly every year, does not provide criteria on which patients should be treated with antibodies against TNF-α, like infliximab.[9] National guidelines however do provide such criteria. Patients should have failed on at least one (Swedish, French and Japanese guidelines) or two (British and Dutch guidelines) disease modifying anti-rheumatic drugs (DMARDs), including methotrexate in an adequate dosage and have a disease activity measured by the Disease Activity Score using 28 joint counts (DAS28)[10] of > 5.1 (British guidelines).[11–16] However, according to the Swedish guidelines no specific disease activity is required for starting with biologicals.[15] The consensus statement of The American College of Rheumatology (ACR) recommends starting with anti-TNF therapy like infliximab in cases with 1) high disease activity (DAS28 > 5.1) for 3 - 6 months, or 2) less than 3 months in combination with features of a poor prognosis (e.g. functional limitation, extra articular disease, rheumatoid factor positivity,

Table 3.1 Summary of reviewed consensus statements and guidelines regarding the use of infliximab

Medical Specialty	Study, year published (reference)	Paper	Country/ Continent
Gastroenterology	*Consensus statements*		
	ECCO, 2006 (6)	European evidence based consensus on the diagnosis and management of Crohn's disease: current management	Europe
	AGA, 2007 (4)	American Gastroenterological Association Consensus Development Conference on the use of biologics in the treatment of inflammatory bowel disease	International
	Guidelines		
	Hommes *et al*, 2006 (7)	Guidelines for treatment with infliximab for Crohn's disease	The Netherlands
	Panaccione *et al*., 2004 (8)	Canadian Association of Gastroenterology clinical practice guidelines: The use of infliximab in Crohn's disease	Canada
Rheumatology	*Consensus statements*		
	Furst *et al*., 2008 (9)	Updated consensus statement on biological agents for the treatment of rheumatic diseases	International
	Braun *et al*., 2006 (18)	First update of the international ASAS consensus statement for the use of anti-TNF agents in patients with ankylosing spondylitis	International
	NVR, 2004 (19)	Statement on the application of TNF-blockade in the treatment of ankylosing spondylitis	The Netherlands
	CRA, 2003 (57)	Canadian rheumatology association consensus on the use of anti-tumor necrosis factor-alpha directed therapies in the treatment of spondyloarthritis	Canada
	Guidelines		
	NICE, 2007 (11)	NICE technology appraisal guidance 130. Adalimumab, etanercept and infliximab for the treatment of rheumatoid arthritis	UK
	NVR, 2003 (12)	Guideline: Application of anti-TNF blockers in the treatment of rheumatoid arthtritis	The Netherlands
	FSR, 2007 (13)	Recommendations of the French Society for Rheumatology regarding TNFalpha antagonist therapy in patients with rheumatoid arthritis	France
	JCR, 2007 (14)	Update on the Japanese guidelines for the use of infliximab and etanercept in rheumatoid arthritis	Japan

Table 3.1 *Continued*

	ACR, 2008 (17)	American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis	USA
	NICE, 2008 (23)	NICE technology appraisal guidance 143. Adalimumab, etanercept and infliximab for ankylosing spondylitis	UK
	NICE, 2007 (24)	NICE technology appraisal guidance 104. Etanercept and infliximab for the treatment of adults with psoriatic arthritis	UK
	BSR, 2005 (16)	Update on the British Society for Rheumatology guidelines for prescribing TNFalpha blockers in adults with rheumatoid arthritis (update of previous guidelines of April 2001)	UK
	BSR, 2005 (21)	BSR guidelines for prescribing TNF-alpha blockers in adults with ankylosing spondylitis. Report of a working party of the British Society for Rheumatology	UK
	FSR, 2007 (22)	Recommendations of the French Society for Rheumatology regarding TNF alpha antagonist therapy in patients with ankylosing spondylitis or psoriatic arthritis: 2007 update	France
Dermatology	*Consensus statements*		
	Reich *et al.*, 2008 (25)	Recommendations for the long-term treatment of psoriasis with infliximab: A dermatology expert group consensus	Europe and Canada
	Sterry *et al.*, 2004 (58)	Biological therapies in the systemic management of psoriasis: International Consensus Conference	International
	Guidelines		
	BAD, 2005 (26)	British Association of Dermatologists guidelines for use of biological interventions in psoriasis 2005	UK
	NVDV, 2005 (27)	Guideline: Application of biologicals in the treatment of psoriasis	The Netherlands
	AAD, 2008 (36)	Guidelines of care for the management of psoriasis and psoriatic arthritis – Section 1. Overview of psoriasis and guidelines of care for the treatment of psoriasis with biologics.	USA
	NICE, 2008 (59)	Infliximab for the treatment of adults with psoriasis	UK
	AAD, 2008 (60)	Guidelines of care for the management of psoriasis and psoriatic arthritis – Section 2. Psoriatic arthritis: Overview and guidelines of care for treatment with an emphasis on the biologics	USA

bony erosions by radiography) or 3) moderate disease activity (DAS28 > 3.2 and < 5.1) for > 6 months and inadequate response to monotherapy with methotrexate in combination with features of poor prognosis.[17]

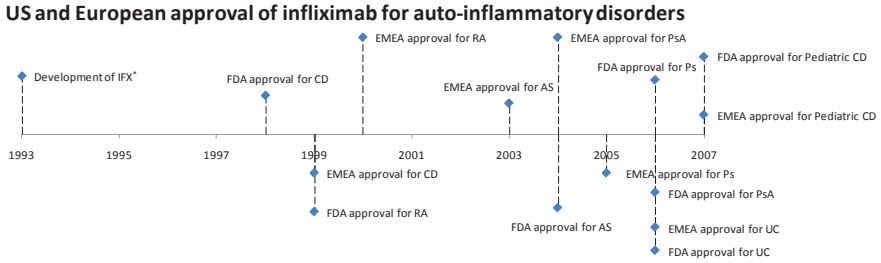

Figure 3.1 Approval by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) of infliximab (IFX). AS, ankylosing spondylitis; CD, Crohn's disease; RA, rheumatoid arthritis, UC, ulcerative colitis; Ps, psoriasis; PsA, psoriatic arthritis. * Knight *et al.*[62]

Ankylosing spondylitis

The international consensus statement from Furst *et al.* does not provide criteria for treatment with infliximab in patients with ankylosing spondylitis.[9] However, another international consensus statement from the ASsessment in Ankylosing Spondylitis (ASAS) working group,[18] as well as a statement from the Dutch Society for Rheumatology[19] gives clear criteria on the use of infliximab in patients who fulfilled the modified New York criteria for the diagnosis of ankylosing spondylitis,[20] including active disease for > 4 weeks, (Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) > 4 (0 - 10) and an expert opinion (the expert should consider clinical features (history and examination), serum acute phase reactant levels and/ or imaging results, such as radiographs demonstrating rapid progression or MRI indicating ongoing inflammation).

Furthermore, all patients should have had adequate therapeutic trials of at least two NSAIDs, which is defined as:

- Treatment for at least 3 months at maximum recommended or tolerated anti-inflammatory dose unless contraindicated
- Treatment for < 3 months where treatment was withdrawn because of intolerance, toxicity or contraindications

The guideline from the French Society for Rheumatology (FSR) is more strict regarding co-medication, stating that patients should have failed at least three NSAIDs used for 3 consecutive months while according to the guidelines from the British Society for Rheumatology (BSR) there should be a failure of conventional treatment with two or more NSAIDs, each taken sequentially at maximum tolerated/ recommended dosage for 4 weeks.[21, 22] Although the guidelines from the BSR recommend treatment with infliximab, the British guidelines from the National Health Service (NHS) state that infliximab is not recommended for the treatment of ankylosing spondylitis.[23]

Psoriatic arthritis

As for rheumatoid arthritis and ankylosing spondylitis, the international consensus statement from Furst *et al.* does not provide criteria for treatment with infliximab in patients with ankylosing spondylitis.[9] According to the NHS guidelines, patients with psoriatic arthritis are eligible for treatment with infliximab in case of peripheral arthritis with three or more tender joints and three or more swollen joints. Furthermore, the psoriatic arthritis has not responded to adequate trials of at least two standard DMARDs, administered either individually or in combination and the patient has been shown to be intolerant of, or have contraindications to, treatment with etanercept or has major difficulties with self administered injections.[24] The FSR guideline is more specific, indicating that the patient must have active and predominantly peripheral disease documented on two occasions at least 4 weeks apart, with both a tender joint count and a swollen joint count of 3 on a total of 76/78 joints and have an overall assessment of disease activity by the physician of 4 on a 10 point scale. Furthermore there should be persistent evidence of active disease after at least 4 months treatment with MTX in a dosage of 15 mg $week^{-1}$, leflunomide 20 mg day^{-1}, or sulfasalazine 2 g day^{-1}.[22]

Dermatology

Few guidelines and consensus statements on the use of infliximab exist for patients with plaque psoriasis. According to the international consensus statement by Reich et al. patients with psoriatic arthritis in association with skin symptoms or moderate to severe psoriasis who have failed two or more systemic therapies are eligible for treatment with infliximab.[25] Furthermore, patients with a Psoriasis Area and Severity Index (PASI) of 20 or patients with an improvement of less than 50% on this scale with previous (non) biological treatment, were eligible for treatment with infliximab.[25] The guideline of the British Association of Dermatologists (BAD) states that patients should have severe disease, defined as a PASI of 10 or more (or a body surface area of 10% or greater where PASI is not applicable) and a Dermatology Life Quality Index >10. Secondly, patients should be unresponsive or intolerant to standard therapy.[26] In the Netherlands, patients are eligible for biological therapies if they have a PASI of 10, and have failed to respond to phototherapy, methotrexate and ciclosporin in the past, or have a contraindication to, or are intolerant to these treatments.[27]

Dosage

The first randomized clinical trial with infliximab (at that time called cA2), in patients with rheumatoid arthritis, randomized patients over a single dose of 1 mg kg^{-1} bodyweight, 10 mg kg^{-1} bodyweight and placebo.[28] In this study, a dosage dependent response was observed. A subsequent study comparing the effect of multiple infusions with infliximab in patients with rheumatoid arthritis compared 1 mg to 3 mg and 10 mg kg^{-1} bodyweight, showing the best results with the latter two.[29] Furthermore it was shown that the median duration of response to the lowest dosage (i.e. 1 mg kg^{-1} bodyweight) lasted 3 weeks, compared with 5 and 8 weeks with dosages of 3 and 10

mg kg^{-1} bodyweight, respectively.[30] Additional studies, performed in patients with Crohn's disease, compared a single dose of 5 mg, 10 mg or 20 mg kg^{-1} bodyweight, administered over a 2 h period. In this trial, patients receiving 5mg kg^{-1} had the best response to infliximab.[31] An open-label trial in Crohn's disease patients, which was performed earlier, compared doses of 1 mg, 5 mg, 10 mg and 20 mg kg^{-1}. The group receiving 1 mg kg^{-1} had a more transient response than the groups given the higher doses.[32] One of the first case reports of psoriasis patients treated with infliximab reported a significant response with 5 mg kg^{-1} bodyweight and the first randomized trial in patients with psoriasis showed significant responses to 5 mg kg^{-1} and 10 mg kg^{-1} bodyweight.[33, 34]

Gastroenterology

With regard to dosing of infliximab in inflammatory bowel disease, as can be seen in table 3.2, international and national consensus statement/guidelines recommend a dosage of 5 mg kg^{-1} body weight given in a 0-2-6-weeks induction regimen followed by maintenance dosing every 8 weeks.[4, 7] The ECCO statement recommends the same dosage, since 5 mg kg^{-1} body weight has been shown effective in large placebo controlled trials.[6, 35] However, this consensus statement gives no information regarding any induction regimen. According to the AGA consensus, primary non response can be determined after two doses.[4] However, the Dutch guidelines recommend assessment of the treatment effect 8 weeks after the third infusion, when infliximab is combined with an immunosuppressant since immunosuppressants such as azathioprine and methotrexate only become effective after about 3 months.[7] When the response is attenuated in patients, dosage can be increased to 10 mg kg^{-1} body weight or the interval between infusions can be shortened up to 4 weeks.[6, 7]

Rheumatology

In patients with rheumatoid arthritis, the standard dosage of infliximab administered recommended by most guidelines is 3 mg kg^{-1} bodyweight in an induction regimen at 0, 2 and 6, and thereafter every 8 weeks.[11, 13, 14] Some of the national and international guidelines do not explicitly state that infliximab should be administered at 3mg kg^{-1} bodyweight, but rather assume that clinicians will administer this 'standard dosage'.[9, 12, 17] As for patients with inflammatory bowel disease, if guidelines refer to attenuation of response, the dosage should be increased or the dosing interval shortened, together with the addition or substitution of another DMARD.[9] The Japanese guideline, however, does not allow any increment of dosage or shortening of interval, and some guidelines do not give recommendations regarding this topic.[12, 14, 17] The National Institute for Clinical Excellence (NICE) guideline is most explicit in its recommendation, recommending increasing the dose of infliximab stepwise by approximately 1.5 mg kg^{-1}, up to a maximum of 7.5 mg kg^{-1} every 8 weeks, or alternatively administering of 3 mg kg^{-1} as often as every 4 weeks.[11] Recommended dosages from the reviewed guidelines and consensus statements regarding the specific diseases as well as the recommended dosage from the manufacturer are given in table 3.2.

Dermatology
The guidelines on the treatment of psoriasis with biologicals from the American Academy of Dermatology (AAD), BAD and the international consensus panel of dermatology experts advises dosing infliximab in a 5 mg kg^{-1} infusion schedule at 0,2 and 6weeks, followed by maintenance treatment every 6 - 8 weeks (table 3.2).[25, 26, 36] The British guidelines however, state that no studies have been performed to establish the optimal dose or frequency of repeated infusions required in order to achieve disease control.[26] The dermatology guidelines give no clear recommendation regarding how to manage attenuated response to infliximab (table 3.2).

Synergy
Repeated administration of infliximab has been associated with immunogenicity, i.e. the formation of antibodies to infliximab (ATI also known as HACA; human anti-chimeric antibodies). The concomitant use of immunosuppressants may increase the efficacy of infliximab, partly because it prevents the development of immunogenicity, and partially by other mechanisms currently unknown.[37–39]

Gastroenterology
The international ECCO guideline has been very clear and advocates that every patient receiving infliximab should receive an immunomodulator (i.e. azathioprine, methotrexate or 6-mercaptopurine) in order to prevent development of antibodies against infliximab that in turn may reduce efficacy and increase side effects.[6] The consensus statement of the AGA strongly recommends co-administration with immunosuppressive therapy as well.[4] The Canadian guidelines are most clear by recommending that all patients, even if they failed to respond to immunomodulators in the past, should receive concomitant immunosuppressants.[8] The Dutch national guideline recommends initiation of immunosuppressants prior to infliximab in order to reduce the formation of antibodies.[7]

Rheumatology
Nearly all efficacy studies with infliximab in rheumatoid arthritis patients have been performed in patients receiving concomitant methotrexate.[29] Therefore, all international, national and local guidelines recommend concomitant treatment with methotrexate in case of starting treatment with any anti TNF-α agent, including infliximab.[13, 17]

Dermatology
The AAD does not recommend concomitant prescription of low-dose methotrexate, although some dermatologists do so to decrease the formation of antibodies.[36] The international consensus statement on the treatment of psoriasis with infliximab does not provide guidelines on the use of concomitant medication and the British guideline states that concomitant systemic therapies may be indicated for some patients with very severe or unstable psoriasis, although doses should be minimized.[25, 26]

Table 3.2 Statements from guidelines and consensus statements for different auto-inflammatory disorders on issues related to the use of infliximab: dosage regimen, induction therapy, loss of response and the use of concomitant medication

Indication	Study, year (reference)	Dosage (mg kg^{-1})	Induction therapy (weeks)	Maintenance intervals (in weeks)	Determination of (non) response*	Advice regarding loss of response in patients who initially responded to IFX	Recommended co-medication
CD	Centocor, 2009 (61)	5	0, 2, 6	8	Active CD: after two doses Fistulizing disease: after three doses	Some patients may regain response with dose escalation	NA
	ECCO, 2006 (6)	5	NA	8	NA	Most try increasing the dose to 10 mg kg^{-1}	AZA, MP or MTX
	AGA, 2007 (4)	5	0, 2, 6	8	After two doses	Patients who have attenuated response may be given • higher dose infusions up to 10 mg kg^{-1} at 8-week intervals, or • 5 mg kg^{-1} at shortened intervals as frequently as every 4 weeks	Initiated in advance of biologic therapy
	Hommes *et al.*, 2006 (7)	5	0, 2, 6	8	4 weeks after the second infusion	Increase to 10 mg kg^{-1} on strict verified indication.	Use of an immunosuppressant.
	Panaccione *et al.*, 2004 (8)	5	0, 2, 6	8	After three doses	Dosage increasement to 10 mg kg^{-1} or shortening of infusion intervals	Concomitant immunosuppressive therapy (eg, 6-MP, AZA or MTX)
UC	Centocor, 2009 (61)	5	0, 2, 6	8	After three doses	NA	NA
	AGA, 2007 (4)	See section on Crohn's disease. No distinction is made in the AGA consensus statement between Crohn's disease and ulcerative colitis					
RA	Furst *et al.*, 2008 (9)	NA	NA	NA	Within 12-24 weeks	Increasing the dose or reducing the dosing intervals may provide additional benefit in RA, as may the addition or substitution of other DMARDs.	MTX
	NVR, 2003 (12)	NA	NA	NA	12 weeks	Increasing dose or reducing the infusion intervals	NA
	JCR, 2007 (14)	3	0, 2, 6	8	NA	Increment of dosage or shortening of interval is not allowed	MTX at a dose of 6–8mg week^{-1}

Table 3.2 *Continued*

	Centocor, 2009 (61)	3	0, 2, 6	8	12 weeks	Options: • Increase the dose step-wise by approximately 1.5 mg kg^{-1}, up to a maximum of 7.5 mg kg^{-1} every 8 weeks or • Administration of 3 mg kg^{-1} as often as every 4 weeks may be considered	MTX
	NICE, 2007 (11)	3	0, 2, 6	8	6 months	Options: • Increase the dose step-wise by approximately 1.5 mg kg^{-1}, up to a maximum of 7.5 mg kg^{-1} every 8 weeks or • Administration of 3 mg kg^{-1} as often as every 4 weeks may be considered.	MTX
	FSR, 2007 (13)	3	0, 2, 6	8	12 weeks	Changes can be made in the dosing interval (every 6 to 8 weeks) or dosage (3 to 5 mg kg^{-1}), or the patient can be switched to another TNF antagonist	MTX or another DMARD
	ACR, 2008 (17)	NA	NA	NA	NA	NA	MTX
	BSR, 2005 (16)	NA	NA	NA	3 months	NA	MTX
AS	Centocor, 2009 (61)	5	0, 2, 6	6 to 8	After two doses	NA	NA
	Furst *et al.*, 2008 (9)	5	0, 2, 6	6 to 8	6-12 weeks	NA	None
	NICE, 2008 (23)	Infliximab is not recommended for the treatment of ankylosing spondylitis					
	FSR, 2007 (22)	NA	NA	NA	6-12 weeks	Changes in dosage or dosing interval or the the patient can be switched to another TNF antagonist	None
	Braun *et al.*, 2006 (18)	5	NA	6 to 8	6-12 weeks	NA	None
	BSR, 2005 (21)	5	0, 2, 6	6 to 8	12 weeks and every 3 months thereafter	NA	NA
	CRA, 2002 (57)	5	0, 2, 6	8	NA	NA	None

Table 3.2 *Continued*

	NVR, 2005 (19)	5	0, 2, 6	6	6–12 weeks and every 6 months thereafter	NA	None
PsA	Centocor, 2009 (61)	5	0, 2, 6	8	NA	NA	MTX
	AAD, 2008 (60)	5	0, 2, 6	6 to 8	NA	Dose and interval of infusion may be adjusted as needed.	NA
	FSR, 2007 (22)	NA	NA	NA	6-12 weeks	Changes in dosage or dosing interval or the the patient can be switched to another TNF antagonist	None
	NICE, 2007 (24)	5	0, 2, 6	8	12 weeks	NA	MTX***
	Furst *et al*., 2008 (9)	NA	NA	NA	NA	NA	NA
Ps	Centocor, 2009 (61)	5	0, 2, 6	8	14 weeks (4 doses)	NA	None
	Reich *et al*., 2008 (25)	5	0, 2, 6	8	12 weeks (or 3 doses)	Decreasing the interval between infusions (e.g. from every 8 weeks to every 6 weeks), increasing the dose of drug administered and/or introducing a supplementary therapy such as a topical treatment or MTX.	
	BAD, 2005 (26)	5	0, 2, 6	8	NA	NA	****
	NVDV, 2005 (27)	3–10 mg kg^{-1}**	0, 2, 6	8	8 weeks	NA	None
	AAD, 2008 (36)	5	0, 2, 6	6 to 8	NA	Dose and interval of infusion may be adjusted as needed	NA
	Sterry *et al.* 2004 (58)	5 or 10	0, 2, 6	NA	NA	NA	NA
	NICE, 2008 (59)	5	0, 2, 6	8	10 weeks	NA	NA

Period after which treatment with IFX should be stopped in case of non-response. **A definitive recommended dose has not been determined yet. ***Following the Summary of Product Characteristics. ****Concomitant systemic therapies may be indicated for some patients with very severe unstable psoriasis, although doses of these should be minimized. AZA, azathioprine; MP, mercaptopurine; MTX, methotrexate; NA, no advice given.

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Monitoring of vital signs

As a foreign protein-derived agent administered intravenously over a 2 h infusion period, infliximab can cause infusion reactions. Formation of antibodies to infliximab may increase the risk of infusion reactions.[37, 39] These infusion reactions can be categorized as acute or delayed. An acute infusion reaction is defined as any adverse event occurring during infusion or within a period of 24 h after infusion.[37, 40] Severity can vary from mild to severe life threatening, and symptoms may include nausea, flushing, dizziness, dyspnoea, chest pain and hypotension or hypertension. Delayed infusion reactions are defined as reactions occurring from 24 h to 14 days after treatment with infliximab and symptoms may include arthralgia, rash, myalgia and fatigue.[37, 40] In randomized controlled trials with infliximab, vital signs (blood pressure, body temperature and pulse) were monitored vigorously. Monitoring body temperature at baseline is performed to rule out fever possibly based on infection and monitoring during infusion is performed while concerns exist about developing fever during an acute infusion reaction. The monitoring of blood pressure and pulse is based on the concern that during infusion with infliximab an anaphylactic shock could develop with typical hypotension.

Gastroenterology

Study protocols with infliximab in inflammatory bowel disease patients and some experts state that 30 min prior to, every 30 min during infusion and up till 2 h after infusion, vital signs (blood pressure, body temperature and pulse) should be monitored.[41] Randomized controlled trials in patients with inflammatory bowel disease reported incidences of acute infusion reactions ranging from 9 - 17%.[35, 42] In clinical practice the overall incidence of acute infusion reactions with infliximab is approximately 4 - 10%.[40, 43] None of the international or national guidelines state that during infusion, vital signs should be monitored. However, in general it is common practice to monitor vital signs during infusion with infliximab.

Rheumatology and dermatology

As in gastroenterology, current practice in rheumatology and dermatology is to monitor vital signs of patients during infusion with infliximab. However, none of the guidelines give specific recommendations regarding monitoring of vital signs.

Interpretation

With the exception of patients treated for rheumatoid arthritis who are treated with a dosage of 3 mg kg^{-1} bodyweight, all patients who are treated with infliximab receive a dosage of 5 mg kg^{-1} bodyweight (table 3.2).To our knowledge, however, randomized controlled trials comparing response rates between 3 mg kg^{-1} or 5 mg kg^{-1} in patients with inflammatory bowel disease, rheumatoid arthritis or psoriasis have not been performed. Klotz *et al.* reviewed the current knowledge on clinical pharmacokinetics of infliximab and stated that little detailed information was available yet and was solely based on measurements of serum concentrations by ELISA using monoclonal

antibodies.[44] Indeed, several studies in patients with rheumatoid arthritis, Crohn's disease, ulcerative colitis, ankylosing spondylitis and psoriasis have shown that there is an interindividual variability of infliximab pharmacokinetics associated with an increase in clinical response with infliximab trough serum concentrations.[45-49] However, in these studies some patients showed good clinical response to infliximab with undetectable serum concentrations of infliximab. Indicating that the correlation between serum concentrations and clinical response is still imprecise. On the other hand, a small observational open label study in patients with rheumatoid arthritis routinely treated with infliximab, showed that the measurement of trough infliximab concentration modified the therapeutic decision for half of their patients and led to improved control of disease activity for patients for whom infliximab dosage was increased.[50] Furthermore with regards to the pharmacokinetics of infliximab, the presence of ATI or HACA, which is associated with an increased risk of infusion reactions and a reduced duration of response, alter the pharmacokinetics of infliximab by an approximately 2.7 fold increase in systemic clearance.[51] Taken together, these findings indicate that further investigations regarding the pharmacokinetics and pharmacodynamics of infliximab are warranted in order to individualize the dosage, based on at least the trough serum concentration and the existence of ATI. Thereby optimizing clinical response and cost effectiveness.

Regarding attenuation of response, the guidelines of each specialty recommend dosage increase or interval shortening or changing to another biological therapy. However, there is no clear recommendation which option should be chosen in which subset of patients. Pharmacokinetic modelling of infliximab in patients with rheumatoid arthritis showed that interval reduction might be more effective in raising serum infliximab concentrations than dosage increase.[46] Flendrie *et al.* observed in an open-label study a more pronounced efficacy in patients with rheumatoid arthritis receiving interval reduction, compared with patients receiving a dosage increase.[52] These observations need to be studied in large randomized trials.

With the exception of ankylosing spondylitis, the need for concomitant administration of immunosuppressants during treatment with infliximab has been stressed by most of the guidelines throughout the different specialties, since it appears to prevent the development of antibodies against infliximab.[37-39] However, benefits and risks of combined strategies should be balanced carefully as the evidence for increased risks of combined therapies is growing. This is most established for serious infections, which is observed in patients with inflammatory bowel disease.[53, 54]

Monitoring of vital signs during infusion with infliximab is based on strict regulations during clinical trials and still advocated in some treatment algorithms and guidelines.[8, 41] We recently showed that scheduled monitoring of vital signs during infusion did neither indicate nor predict development of acute infusion reactions.[55] When baseline vital signs from patients with and without acute infusion reactions were compared, no significant differences were observed. Furthermore, during an acute infusion reaction, vital signs did not show a significant change compared with baseline.[55]

Conclusion

By reviewing current guidelines and consensus statements within the medical specialties of rheumatology, gastroenterology and dermatology on the use of infliximab for auto-inflammatory disorders, several topics (i.e. dosage of infliximab, monitoring of vital signs, use of concomitant medication and loss of response) were discussed and shortcomings in guidelines and consensus statements regarding these topics were identified. Based on this discussion, several recommendations have been made, as can be seen in table 3.3. Finally, as stressed by a recent quality appraisal of clinical practice guidelines and consensus statements on the use of biological agents in rheumatoid arthritis, guidelines should be explicit in their guidance,[56] which has implications for the development of future guidelines.

Table 3.3 Conclusions and recommendations

Topics	Conclusions and recommendations
Dosage of infliximab	Based on several controlled clinical studies, certain standard dosage regimens for infliximab have been defined which probably need some re-evaluation in terms of improving benefit: risk ratios.[44]
	Future studies are needed to study the pharmacokinetic–pharmacodynamic relationship of infliximab as a necessary step before therapeutic drug monitoring can be recommended in guidelines.
Monitoring vital signs	Routine scheduled measurement of vital signs during infusion is not valuable in detecting acute infusion reactions and should only be performed in the case of an acute infusion reaction.[55]
	We recommend to administer infliximab at an infusion unit under supervision of trained personnel. This approach enables direct interventions in case a patient reports symptoms. Baseline assessment of patients, including vital signs, should still be performed as normal clinical practice to rule out possible infections or other contraindications for infusion with infliximab.
Use of concomitant medication	Efforts should be made to establish a reasonable time interval in which concomitant medication should be decreased.
Loss of response to infliximab	Although some evidence exists that interval reduction might be more effective in raising serum infliximab concentrations than dosage increase, large randomized trials are needed to observe whether or not interval reduction is superior to dosage increase. Furthermore, in which subset of patients in order to be able to give guidance regarding loss of response in clinical guidelines.

References

1. Lowes MA, Bowcock AM, Krueger JG. Pathogenesis and therapy of psoriasis. Nature 2007;445:866-873.
2. Scott DL, Kingsley GH. Tumor necrosis factor inhibitors for rheumatoid arthritis. N Engl J Med 2006;355:704-712.
3. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448:427-434.
4. Clark M, Colombel JF, Feagan BC, et al. American Gastroenterological Association Consensus Development Conference on the use of biologics in the treatment of inflammatory bowel disease, June 21–23, 2006. Gastroenterology 2007;133:312–339.
5. Lichtenstein GR, Yan SK, Bala M, et al. Infliximab maintenance treatment reduces hospitalizations, surgeries, and procedures in fistulizing Crohn's disease. Gastroenterology 2005;128:862-869.
6. Travis SPL, Stange EF, Lemann M, et al. European evidence based consensus on the diagnosis and management of Crohn's disease: current management. Gut 2006;55:i16-i35.
7. Hommes DW, Oldenburg B, van Bodegraven AA, et al. Guidelines for treatment with infliximab for Crohn's disease. Neth J Med 2006; 64: 219-29.
8. Panaccione R, Fedorak RN, Aumais G, et al. Canadian Association of Gastroenterology clinical practice guidelines: the use of infliximab in Crohn's disease. Can J Gastroenterol 2004;18:503–508.
9. Furst DE, Keystone EC, Kirkham B, et al. Updated consensus statement on biological agents for the treatment of rheumatic diseases, 2008. Ann Rheum Dis 2008;67:2–25.
10. Prevoo MLL, Vanthof MA, Kuper HH, et al. Modified disease-activity scores that include 28-joint counts – development and validation in a prospective longitudinal-study of patients with rheumatoid-arthritis. Arthritis Rheum 1995;38:44–48.
11. Dillon A, on behalf of the National Institute for Health and Clinical Excellence. Adalimumab, etanercept and infliximab for the treatment of Rheumatoid arthritis. October 2007. Available at http://www.nice.org.uk/nicemedia/pdf/TA130guidance.pdf (last accessed 17 August 2010).
12. Medicijnen: Het toepassen van TNF blokkade in de behandeling van reumatoide arthritis. November 2003. Available at http://www.nvr.nl/uploads/51/320/NVR_Medicijnen_richtlijn_TNF-RA.pdf (last accessed 17 August 2010).
13. Fautrel B, Pham T, Mouterde G, et al. Recommendations of the French Society for Rheumatology regarding TNFalpha antagonist therapy in patients with rheumatoid arthritis. Joint Bone Spine 2007;74:627-37.
14. Koike R, Takeuchi T, Eguchi K, et al. Update on the Japanese guidelines for the use of infliximab and etanercept in rheumatoid arthritis. Mod Rheumatol 2007;17:451-458.
15. Soderlin MK, Geborek P. Changing pattern in the prescription of biological treatment in rheumatoid arthritis. A 7-year follow-up of 1839 patients in southern Sweden. Ann Rheum Dis 2008;67:37-42.
16. Ledingham J, Deighton C. Update on the British Society for Rheumatology guidelines for prescribing TNF alpha blockers in adults with rheumatoid arthritis (update of previous guidelines of April 2001). Rheumatology 2005;44:157-163.
17. Saag KG, Teng GG, Patkar NM, et al. American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis. Arthritis Rheum 2008;59:762-784.

18. Braun J, Davis J, Dougados M, et al. First update of the international ASAS consensus statement for the use of anti-TNF agents in patients with ankylosing spondylitis. Ann Rheum Dis 2006;65:316-320.
19. Van der Horst-Bruinsma IE, Franssen MJAM, Oostveen JCM, et al. Richtlijn voor de diagnostiek en behandeling van spondylitis ankylopoetica 2009. Available at http// www.nvr.nl/uploads/237/131/NVR_Reumatische_ziekten_richtlijn_Bechterew.pdf (last accessed 17 August 2010).
20. Vanderlinden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis – A proposal for modification of the New York criteria. Arthritis Rheum 1984; 27:361-368.
21. Keat A, Barkham N, Bhalla A, et al. BSR guidelines for prescribing TNF-alpha blockers in adults with ankylosing spondylitis. Report of a working party of the British Society for Rheumatology. Rheumatology 2005;44:939-947.
22. Pham T, Fautrel B, Dernis E, et al. Recommendations of the French Society for Rheumatology regarding TNF alpha antagonist therapy in patients with ankylosing spondylitis or psoriatic arthritis: 2007 update. Joint Bone Spine 2007;74:638-646.
23. Dillon A, on behalf of the National Institute for Health and Clinical Excellence. Adalimumab, etanercept and infliximab for ankylosing spondylitis.May 2008. Available at http://www.nice.org.uk/nicemedia/live/11992/40761/40761.pdf (last accessed 17 August 2010).
24. Dillon A, on behalf of the National Institute for Health and Clinical Excellence. Etanercept and infliximab for the treatment of adults with psoriatric arthritis. 2007. Available at http://www.nice.org.uk/nicemedia/live/11582/33404/33404.pdf (last accessed 17 August 2010).
25. Reich K, Griffiths C, Barker J, et al. Recommendations for the long-term treatment of psoriasis with infliximab: a dermatology expert group consensus. Dermatology 2008;217:268-275.
26. Smith CH, Anstey AV, Barker JN, et al. British Association of Dermatologists guidelines for use of biological interventions in psoriasis 2005. Br J Dermatol 2005;153:486-497.
27. Nederlandse vereniging voor dermatologie en venereologie. Het toepassen van biologicals in de behandeling van patiënten met plaque psoriasis. December 2004. Available at http://www.huidziekten.nl/richtlijnen/nvdvbiologicals2005concept.doc (last accessed 17 August 2010).
28. Elliott MJ, Maini RN, Feldmann M, et al. Randomized double-blind comparison of chimeric monoclonal-antibody to tumor-necrosisfactor-alpha (Ca2) versus placebo in rheumatoid-arthritis. Lancet 1994;344:1105-1110.
29. Maini RN, Breedveld FC, Kalden JR, et al. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum 1998;41:1552–1563.
30. Maini RN, Elliot MJ, Longfox A, et al. Clinical-response of rheumatoid arthritis (Ra) to anti-TNF-alpha (Ca2) monoclonal antibody (Mab) is related to administered dose and persistence of circulating antibody. Arthritis Rheum 1995;38:200.
31. Targan SR, Hanauer SB, van Deventer SJ, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. N Engl J Med 1997;337:1029-1035.
32. McCabe RP,Woody J, van Deventer S, et al. A multicenter trial of cA2 anti-TNF chimeric monoclonal antibody in patients with active Crohn's disease. Gastroenterology 1996;110: A962.

33. Oh CJ, Das KM, Gottlieb AB. Treatment with anti-tumor necrosis factor alpha (TNF-alpha) monoclonal antibody dramatically decreases the clinical activity of psoriasis lesions. J Am Acad Dermatol 2000;42:829-830.
34. Chaudhari U, Romano P, Mulcahy LD, et al. Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: a randomised trial. Lancet 2001;357:1842-1847.
35. Hanauer SB, Feagan BG, Lichtenstein GR, et al. ACCENT I Study Group.Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet 2002; 359: 1541–9.
36. Menter A, Gottlieb A, Feldman SR, et al. Guidelines of care for the management of psoriasis and psoriatic arthritis – Section 1. Overview of psoriasis and guidelines of care for the treatment of psoriasis with biologics. J Am Acad Dermatol 2008;58:826-850.
37. Baert F, Noman M, Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med 2003;348:601-608.
38. Wolbink GJ, Vis M, Lems W, et al. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. Arthritis Rheum 2006;54:711-715.
39. de Vries MK,Wolbink GJ, Stapel SO, et al. Inefficacy of infliximab in ankylosing spondylitis is correlated with antibody formation. Ann Rheum Dis 2007;66:133-134.
40. Cheifetz A, Smedley M, Martin S, et al. The incidence and management of infusion reactions to infliximab: a large center experience. Am J Gastroenterol 2003;98:1315-1324.
41. Sandborn WJ, Hanauer SB. Infliximab in the treatment of Crohn's disease: a user's guide for clinicians.Am J Gastroenterol 2002;97:2962-2972.
42. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med 2005;353:2462-2476.
43. Colombel JF, Loftus EV Jr, Tremaine WJ, et al. The safety profile of infliximab in patients with Crohn's disease: the Mayo Clinic experience in 500 patients. Gastroenterology 2004;126:19-31.
44. Klotz U, Teml A, Schwab M. Clinical pharmacokinetics and use of infliximab. Clin Pharmacokinet 2007;46:645-660.
45. Seow CH, Newman A, Irwin SP, et al. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. Gut 2010;59:49-54.
46. St Clair EW, Wagner CL, Fasanmade AA, et al. The relationship of serum infliximab concentrations to clinical improvement in rheumatoid arthritis – Results from ATTRACT, a multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2002;46:1451-1459.
47. Maser EA, Villela R, Silverberg MS, et al. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. Clin Gastroenterol Hepatol 2006;4:1248-1254.
48. Reich K, Nestle FO, Papp K, et al. Infliximab induction and maintenance therapy for moderate-to-severe psoriasis: a phase III, multicentre, double-blind trial. Lancet 2005;366:1367-1374.
49. de Vries MK,Wolbink GJ, Stapel SO, et al. Decreased clinical response to infliximab in ankylosing spondylitis is correlated with anti-infliximab formation. Ann Rheum Dis 2007;66:1252-1254.
50. Mulleman D, Méric JC, Paintaud G, et al. Infliximab concentration monitoring improves the control of disease activity in rheumatoid arthritis. Arthritis Res Ther 2009;11:R178.
51. Ternant D, Aubourg A, Magdelaine-Beuzelin C, et al. Infliximab pharmacokinetics in inflammatory bowel disease patients. Ther Drug Monit 2008;30:523-529.

52. Flendrie M, Creemers MCW, Van Riel PLCM. Titration of infliximab treatment in rheumatoid arthritis patients based on response patterns. Rheumatology 2007;46:146-149.
53. Lichtenstein GR, Feagan BG, Cohen RD, et al. Serious infections and mortality in association with therapies for Crohn's disease: TREAT registry. Clin Gastroenterol Hepatol 2006;4:621-630.
54. Fidder H, Schnitzler F, Ferrante M, et al. Long-term safety of infliximab for the treatment of inflammatory bowel disease: a single-centre cohort study.Gut 2009;58:501-508.
55. de Vries HS, van Oijen MGH, van Hoven-van Loo KEJ, et al. Monitoring vital signs during infusion with infliximab does neither indicate nor predict development of acute infusion reactions. J Clin Gastroenterol 2009;43:387-388.
56. Lopez-Olivo MA, Kallen MA, Ortiz Z, et al. Quality appraisal of clinical practice guidelines and consensus statements on the use of biologic agents in rheumatoid arthritis: a systematic review. Arthritis Rheum-Arthritis Care Res 2008;59:1625-1638.
57. Maksymowych WP, Inman RD, Gladman D, et al. Canadian Rheumatology Association consensus on the use of anti-tumor necrosis factor-alpha directed therapies in the treatment of spondyloarthritis. J Rheumatol 2003;30:1356-1363.
58. Sterry W, Barker J, Boehncke WH, et al. Biological therapies in the systemic management of psoriasis: international consensus conference. Br J Dermatol 2004;151:3–17.
59. Dillon A, on behalf of the National Institute for Health and Clinical Excellence. Infliximab for the treatment of adults with psoriasis. January 2008. Available at http://www.nice.org.uk/nicemedia/live/11910/38954/38954.pdf (last accessed 17 August 2010).
60. Gottlieb A, Korman NJ, Gordon KB, et al. Guidelines of care for the management of psoriasis and psoriatic arthritis – Section 2. Psoriatic arthritis: Overview and guidelines of care for treatment with an emphasis on the biologics. J Am Acad Dermatol 2008;58:851-864.
61. Centocor Product Information. 2009. Availalble at http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000240/WC500050888.pdf (last accessed 17 August 2010).
62. Knight DM, Trinh H, Le J, et al. Construction and initial characterization of a mouse-human chimeric anti-TNF antibody. Mol Immunol 1993;30:1443–1453.

Chapter 4

Infliximab exerts no direct hepatotoxic effect on HepG2 cells *in vitro*

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Infliximab-induced hepatotoxicity is reported in several case studies involving patients with inflammatory bowel disease (IBD) and a direct hepatotoxic effect has been proposed. The aim of this study was to determine the direct *in vitro* toxicity of infliximab. As a proof of principle the *in vitro* toxicity of thiopurines and methotrexate was also determined.

Cell survival curves and the half maximal inhibitory concentrations (IC_{50}) were obtained after 24, 48 and 72 hours of incubation in HepG2 cells with the IBD drugs azathioprine, 6-mercaptopurine, 6-thioguanine, methotrexate or infliximab by using the WST-1 cytotoxicity assay.

No *in vitro* hepatotoxicity was seen with infliximab, while concentration-dependent cytotoxicity was observed when HepG2 cells were incubated with increasing concentrations of azathioprine, 6-mercaptopurine and 6-thioguanine. Infliximab alone or given in combination with azathioprine showed no direct hepatotoxic effect *in vitro*, indicating that the postulated direct hepatotoxicity of infliximab is unlikely.

Introduction

Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to a drug or another noninfectious agent.[1] It is a serious complication, frequently observed in the medical treatment of inflammatory bowel disease (IBD).[2] It can be directly attributed to the type of drugs used to treat IBD, such as immunosuppressants or biological therapies targeting TNF-α. However, hepatotoxicity may also result from drugs used to treat complications of immunosuppressants and TNF-α antagonists, e.g. isoniazid for treatment of reactivation tuberculosis, or may be due to exacerbation of underlying chronic viral hepatitis caused by immunosuppression.[3]

In December 2004 the Food and Drug Administration (FDA) issued a drug warning to alert health care professionals on the risk of hepatotoxicity linked to infliximab.[4] However, severe hepatic reactions, including acute liver failure, jaundice, hepatitis or cholestasis have been rarely reported in patients receiving infliximab.[5-8] Furthermore, as demonstrated by Sokolove *et al.* elevations of serum transamines in patients receiving biological therapy are uncommon and abnormalities of more than two times the upper limit of normal are rarely observed.[6] The mechanism of infliximab-induced hepatotoxicity is poorly understood, although a direct hepatoxic effect has been proposed by several authors.[6-8] To our knowledge, no in vivo or *in vitro* results supporting this hypothesis have been reported.

The aim of this study was to determine the *in vitro* hepatotoxicity of infliximab. As a proof of principle, conventional IBD medication i.e. the thiopurines azathioprine, 6-mercaptopurine and 6-thioguanine and methotrexate, which all are known to be hepatotoxic, were also tested. Although cultures of primary human hepatocytes seem to have the most relevant physiological properties for the evaluation of in vitro IBD drug hepatotoxicity, they are difficult to obtain and rapidly lose their metabolic properties.[9] Therefore, we used a human liver hepatocellular carcinoma (HepG2) cell line, which is very stable, easy to handle and previously used in drug toxicity studies.[9] HepG2 cells were incubated with increasing concentrations of infliximab, methotrexate or thiopurines for 24, 48 or 72 hours and subsequently cell viability was determined.

Materials & Methods

Cell culture

Human hepatocellular carcinoma (HepG2) cells (American Type Culture Collection, Rockville, Maryland, USA) were grown in Dulbecco's Modified Eagle Medium (DMEM, PAA Laboratories GmbH, Pasching, Austria) containing 10% (v/v) heat-inactivated fetal bovine serum (Gibco Invitrogen, Paisley, Scotland), 1X non-essential amino acids (PAA), 20mM HEPES buffer (PAA) and 50 mg/l gentamycin (Gibco) at 37 ^{0}C in a humidified atmosphere containing 5% CO_2. Medium was renewed every 3 days and when confluence was reached, cells were harvested with trypsin/EDTA

(Cambrex, Verviers, Belgium), washed with phosphate-buffered saline, and used for cytotoxicity assays.

Cytotoxicity assays
HepG2 cells were seeded in flat-bottomed 96-well microtitre plates (Costar; Corning Inc., Corning, New York, USA) at a density of 5.0 x 10^4 cells per well in a final volume of 100 µl culture medium, and cells were cultured for 24 hours. Subsequently, cells were incubated with the single drugs azathioprine, 6-mercaptopurine, methotrexate (all Sigma-Aldrich Chemie B.V., Zwijndrecht, the Netherlands), 6-thioguanine (Alfa Aesar GmbH & Co KG, Karlsruhe, Germany) or infliximab (Remicade©, Centocor, Leiden, the Netherlands), for 24, 48 and 72 hours. The following concentration series were used; azathioprine: 0.002 µM - 4 mM; 6-mercaptopurine: 0.002 µM - 200 µM; 6-thioguanine: 0.002 µM-4 mM; methotrexate: 50 nM-100 µM; infliximab: 0.002 mg/l - 5 g/l.

In subsequent experiment cytotoxicity of a combinations of drugs was tested. A single, non-toxic concentration of azathioprine (1µM) was tested in combination with a concentration range of infliximab (0.002 mg/l - 5 g/l), whereas a single, non-toxic concentration of infliximab (312 mg/l) was tested in combination with a concentration range of azathioprine (0.002 µM - 4 mM).

All drugs were first dissolved in 0.1 M NaOH and then rapidly diluted in culture medium to reach the final concentration. Refreshment of culture medium with the various concentrations of drugs was done every 24 hours. After incubation with drugs, cell survival assessment was performed by adding water-soluble tetrazolium salt-1 (WST-1, Roche Diagnostics Nederland BV, Almere, the Netherlands) according to the manufacturer's instructions. In the WST-1 assay, tetrazolium salts are cleaved by dehydrogenases of viable cells to produce formazan. The amount of formazan dye is quantified by using an ELISA plate reader at 440 nm. Cell survival was defined as: Cell survival = $(A_{experimental} - A_{background})/(A_{control} - A_{background})$ x 100%, with $A_{experimental}$ being the absorbance of drug incubated cells plus WST-1, $A_{background}$ being the absorbance of culture medium plus WST-1 in the absence of cells and $A_{control}$ being the absorbance of cells without drugs plus WST-1. Test results were obtained from three independent experiments, each performed in triplicate.

Statistics
One way ANOVA analysis was used to compare the effect of incubation-time. A p-value < 0.05 was considered statistically significant. Data were normalized to untreated cells using GraphPad Prism, to calculate the concentration at which cell survival is 50% (IC_{50}). All statistical analyses and calculations were carried out using GraphPad Prism (version 4.0; GraphPad Software, San Diego, California, USA).

Results

The incubation of HepG2 cells with increasing concentrations of infliximab did not result in significant changes in cell viability, indicating that a direct in vitro cytotoxic effect was absent (figure 4.1). At all concentrations of infliximab tested after 24, 48 or 72 hours of incubation, cell survival was above 50% and no IC_{50} values could be determined (table 4.1). Only at the highest concentration of 5mg/l, could a decrease of about 30% in cell survival be seen (figure 4.1). Concentration-dependent cytotoxicity was clearly observed when HepG2 cells were incubated with increasing concentrations of azathioprine, 6-mercaptopurine or 6-thioguanine (figure 4.2). Only methotrexate showed a time-dependent cytotoxic effect on HepG2 cells. Incubation with various concentrations of methotrexate for 72 hours resulted in a significant difference in survival when compared to 24 hours of incubation ($p < 0.01$).
After 24 hours incubation, neither the combination of infliximab (0.002 mg/l - 5 g/l) with a low dose (1 μM) of azathioprine nor the combination of a single, non-toxic concentration of infliximab (312 mg/l) in combination with azathioprine (0.002 μM - 4 mM) did show any difference in cell survival (data not shown).

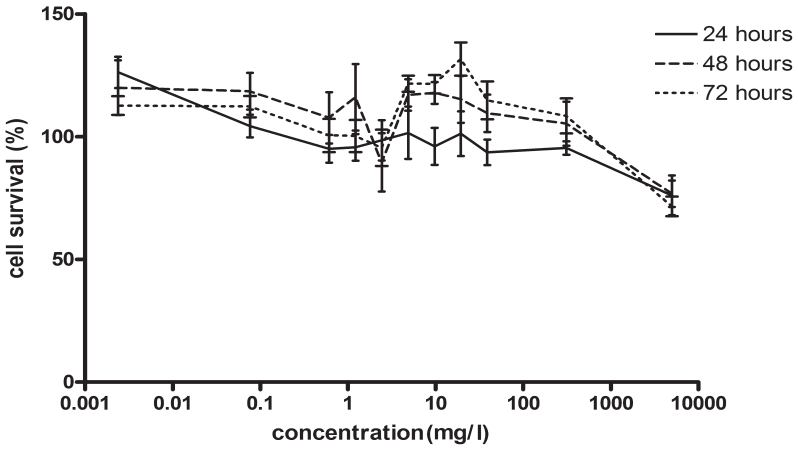

Figure 4.1: Cell survival of HepG2 cells after exposure to various concentrations of infliximab for 24, 48 or 72 hours. Cell survival was expressed as a percentage of untreated cells. Values are means ± SEM of three independent experiments performed in triplicate.

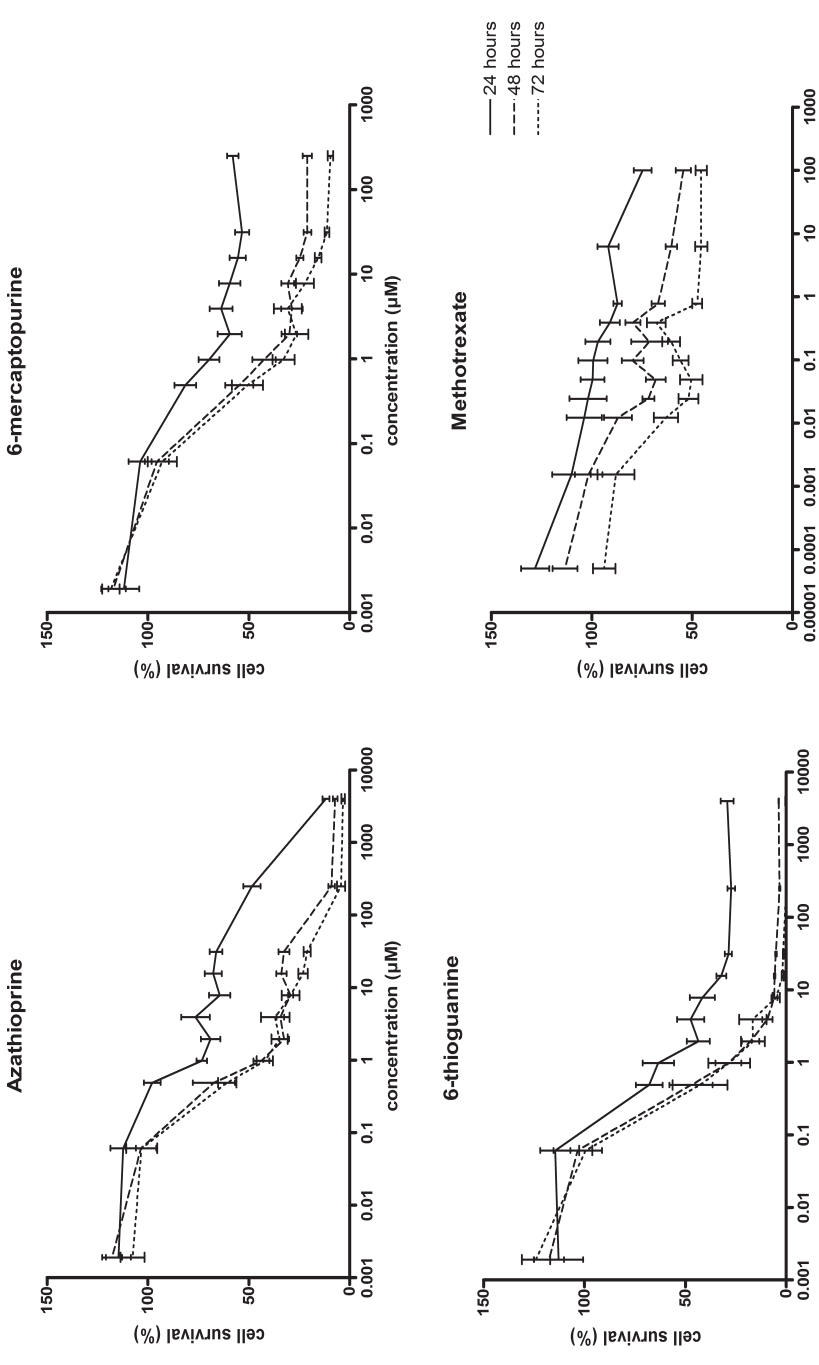

Figure 4.2: Effect of thiopurines and methotrexate on HepG2 cell viability. HepG2 cells were incubated with various concentrations of IBD drugs for 24, 48 or 72 hours and cell survival was measured and expressed as percentage of untreated cells. The graphs summarize the results of three independent experiments (means ± SEM), performed in triplicate.

Table 4.1 Cytotoxicity of IBD drugs in HepG2 cells.

	IC_{50} [μM]				
	Azathioprine	6-Mercaptopurine	6-Thioguanine	Methotrexate	Infliximab
24 hours	33.7 ± 29.3	3.8 ± 4.1	1.1 ± 0.8	NA	NA
48 hours	0.5 ± 0.3	0.5 ± 0.6	0.4 ± 0.5	9.3 ± 16.0	NA
72 hours	0.9 ± 0.6	0.4 ± 0.3	0.5 ± 0.7	0.04 ± 0.03	NA

Values are mean IC50 ± SD (n = 9) derived from after normalization of dose response curves using Graphpad Prism. NA; not applicable (IC_{50} value was not reached)

Discussion

In this study infliximab showed no direct cytotoxic effect on HepG2 cells, even at concentrations far exceeding the maximum concentration of 118 μg/ml, which infliximab achieves when administered at a dosage of 5 mg/kg IV.[10] Concomitant incubation with both infliximab in different dosages and azathioprine at a non toxic concentration did not alter HepG2 cell viability. Our *in vitro* results therefore suggest that a direct hepatotoxicity of infliximab is implausible. Alternatively, infliximab-induced hepatotoxicity is more likely to be immuno-mediated or induced via Fc receptor-mediated interactions. After forming an immune complex with TNF-α, this complex is cleared by the mononuclear phagocytic system in the liver via Fc receptor-mediated interactions that in turn can activate Kupffer cells. These resident macrophages of the liver located in hepatic sinusoids, do release reactive oxygen species which may lead to local damage of hepatocytes.[11-13] During infliximab therapy, increased formation of anti-nuclear antibodies has been observed,[14] most possible due to the fact that binding of infliximab to transmembrane TNF on the cell surface induces apoptosis, leading to the release of nucleosomes and generation of anti-nuclear antibodies.[15] Since antibodies to TNF-α delay the repair of liver injury,[16, 17] the use of infliximab might also exacerbate a previous suboptimal liver condition not recognized by any clinical symptoms or biochemical markers. Furthermore, a potential hepato-protective effect of TNF-α induced by increasing hepatocyte regeneration and decreasing apoptosis has been observed in a transgenic mouse model of chronic hepatitis C while treatment with anti-TNF-α blocked the anti-apoptotic and regenerative effects induced by TNF-α.[18]

In contrast to our experience with infliximab, we observed a concentration dependent cytotoxic effect of the thiopurines in HepG2 cells, while methotrexate demonstrated a time- and concentration-dependent effect. The *in vitro* hepatotoxic effects of thiopurines have also been demonstrated by Petit *et al.*, comparing the cytotoxicity of thiopurines in human hepatocytes and HepaRG cells, incubated for 24, 48, 72 and 96 hours with 1, 5 or 25 μM of azathioprine, 6-mercaptopurine or 6-thioguanine. They reported a dose- and time-dependent cytotoxic effect of azathioprine and 6-mercaptopurine in both human hepatocytes and HepRG cells, while 6-thioguanine

had no significant effect on human hepatocytes. However, 72 hours of incubation with either 5 or 25 μM of 6-thioguanine showed a 30% decrease in cell survival of HepaRG cells.[19]

The observed time-dependent cytotoxic effect of methotrexate in our study is in line with results of Yin *et al.* who reported a time- and concentration dependent effect of high dose methotrexate (1 - 10 mM) in rat hepatocytes.[20] These concentrations however go far beyond the mean peak concentration in human plasma of 1.14 μM achieved after subcutaneous administration of 15 mg methotrexate to patients with IBD.[21]

Several limitations of our study should be noticed. First of all, results of *in vitro* studies cannot be directly extrapolated to the *in vivo* situation. Isolated liver (carcinoma) cells will respond differently to stress or toxic compounds than to an intact and perfused liver. Therefore, although results from cell lines add to the understanding of drug-induced toxicity, they will be difficult to translate into clinical practice. Processes of absorption, distribution, metabolism and excretion, which determine the exposure of the target tissues of an organism *in vivo*, are mainly absent in *in vitro* studies.[22] Furthermore, peripheral serum or plasma concentrations do not reflect the concentrations in the portal vein. Therefore the drug concentrations to which the liver is exposed are largely unknown. These points are especially true for the thiopurines. Resorption is highly variable in animal studies as well as in patients with IBD and the pro-drugs azathioprine and 6-mercaptopurine are rapidly converted.[23-26] Additionally, as in hepatocytes in the primary culture, changes in the expression of drug metabolizing enzymes over time occur in HepG2 cells.[27] Therefore neither primary cultures of hepatocytes nor HepG2 cells display an ideal model mimicking the expression levels of drug metabolizing enzymes as present in hepatocytes *in vivo*, thereby limiting the reproducibility of *in vitro* hepatotoxicity experiments using different cell cultures.[27] In our study we focused on cell viability as a marker of hepatotoxicity. Alterations in pathways underlying cell death including oxidative stress were not studied.

In conclusion, our study suggest that infliximab does not have a direct toxic effect on HepG2 cells. In addition, infliximab in combination with thiopurines does not increase their *in vitro* toxicity on HepG2 cells. Our results may not be translated to clinical practice directly without considering the limitations of these findings. On the other hand, no alarming cytotoxicity is seen in the same assay that shows evident dose-related thiopurine cytotoxicity. Future studies regarding the hepatotoxic effects of infliximab should focus on Fc receptor-mediated interactions and auto-immune related factors.

References

1. Navarro VJ, Senior JR. Drug-related hepatotoxicity. N Engl J Med 2006;354:731-739.
2. Rogler G. Gastrointestinal and liver adverse effects of drugs used for treating IBD. Best Pract Res Clin Gastroenterol 2010;24:157-165.
3. Khokhar OS, Lewis JH. Hepatotoxicity of agents used in the management of inflammatory bowel disease. Dig Dis 2010;28:508-518.
4. http://www.fda.gov/medWatch/safety/2004/remicade_DHCP_dec04.pdf
5. http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/103772s5234lbl.pdf
6. Sokolove J, Strand V, Greenberg JD, et al. Risk of elevated liver enzymes associated with TNF inhibitor utilisation in patients with rheumatoid arthritis. Ann Rheum Dis 2010;69:1612-1617.
7. Mancini S, Amorotti E, Vecchio S, et al. Infliximab-related hepatitis: discussion of a case and review of the literature. Intern Emerg Med 2010;5:193-200.
8. Ierardi E, Valle ND, Nacchiero MC, et al. Onset of liver damage after a single administration of infliximab in a patient with refractory ulcerative colitis. Clin Drug Investig 2006;26:673-676.
9. Yeon JH, Na D, Park JK. Hepatotoxicity assay using human hepatocytes trapped in microholes of a microfluidic device. Electrophoresis 2010;31:3167-3174.
10. Tracey D, Klareskog L, Sasso EH, et al. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. Pharmacol Ther 2008;117:244-279.
11. Johansson AG, Sundqvist T, Skogh T. IgG immune complex binding to and activation of liver cells. An in vitro study with IgG immune complexes, Kupffer cells, sinusoidal endothelial cells and hepatocytes. Int Arch Allergy Immunol 2000; 121:329-336.
12. Aithal GP. Hepatotoxicity related to antirheumatic drugs. Nat Rev Rheumatol 2011; 7:139-150.
13. Rojas JR, Taylor RP, Cunningham MR, et al. Formation, Distribution, and Elimination of Infliximab and Anti-Infliximab Immune Complexes in Cynomolgus Monkeys. J Pharmacol Exp Ther 2005; 313:578-585.
14. Ferraro-Peyret C, Coury F, Tebib JG, et al. Infliximab therapy in rheumatoid arthritis and ankylosing spondylitis-induced specific antinuclear and antiphospholipid autoantibodies without autoimmune clinical manifestations: a two-year prospective study. Arthritis Res Ther 2004; 6:R535-543.
15. D'Auria F, Rovere-Querini P, Giazzon M, et al. Accumulation of plasma nucleosomes upon treatment with anti-tumour necrosis factor-alpha antibodies. J Intern Med. 2004; 255:409-418.
16. Akerman P, Cote P, Yang SO, et al. Antibodies to tumor necrosis factor-alpha inhibit liver regeneration after partial hepatectomy. Am J Physiol 1992;263:G579-585.
17. Bruccoleri A, Gallucci R, Germolec DR, et al. Induction of early-immediate genes by tumor necrosis factor alpha contribute to liver repair following chemical-induced hepatotoxicity. Hepatology 1997;25:133-141.
18. Brenndorfer ED, Weiland M, Frelin L, et al. Anti-tumor necrosis factor α treatment promotes apoptosis and prevents liver regeneration in a transgenic mouse model of chronic hepatitis C. Hepatology 2010;52:1553-1563
19. Petit E, Langouet S, Akhdar H, et al. Differential toxic effects of azathioprine, 6-mercaptopurine and 6-thioguanine on human hepatocytes. Toxicol In Vitro 2008;22:632-642.

20. Yin J, Meng Q, Zhang G, et al. Differential methotrexate hepatotoxicity on rat hepatocytes in 2-D monolayer culture and 3-D gel entrapment culture. Chem Biol Interact 2009;180:368-375.
21. Egan LJ, Sandborn WJ, Mays DC, et al. Systemic and intestinal pharmacokinetics of methotrexate in patients with inflammatory bowel disease. Clin Pharmacol Ther 1999;65:29-39.
22. Tostmann A, Boeree MJ, Peters WHM, et al. Isoniazid and its toxic metabolite hydrazine induce in vitro pyrazinamide toxicity. Int J Antimicrob Agents 2008;31:577-580.
23. Zimm S, Collins JM, Riccardi R, et al. Variable bioavailability of oral mercaptopurine: is maintenance chemotherapy in acute lymphoblastic leukemia being optimally delivered? N Engl J Med. 1983;308:1005-1009.
24. Ding TL, Benet LZ. Comparative bioavailability and pharmacokinetic study of azathioprine and 6-mercaptopurine in the rhesus monkey. Drug Metab Dispos. 1979;7:373-377.
25. Deibert P, Dilger K, Fischer C, et al. High variation of tioguanine absorption in patients with chronic active Crohn's disease. Aliment Pharmacol Ther. 2003;18:183-189.
26. Chan GL, Canafax DM, Johnson CA. The therapeutic use of azathioprine in renal transplantation. Pharmacotherapy. 1987;7:165-177.
27. Wilkening S, Bader A. Influence of Culture Time on the Expression of Drug-Metabolizing Enzymes in Primary Human Hepatocytes and Hepatoma Cell Line HepG2. J Biochem Mol Toxicol 2003;17:207-213.

Chapter 5

A functional polymorphism in *UGT1A1* related to hyperbilirubinemia is associated with a decreased risk for Crohn's disease

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An imbalance between the production of reactive oxygen species (ROS) and their capturing by antioxidants results in oxidative stress. This may play an important role in the pathogenesis of inflammatory bowel disease (IBD). Since bilirubin is an important endogenous antioxidant, increased levels of bilirubin may protect against IBD. UDP-glucuronosyltransferase 1A1 (UGT1A1) is the only enzyme involved in the conjugation of bilirubin and the common *UGT1A1*28* allele in the *UGT1A1* gene, which is strongly associated with Gilbert's syndrome in Caucasians, results in elevated plasma bilirubin levels.

We tested the hypothesis that the *UGT1A1*28* allele is associated with lower disease susceptibility to, and disease behavior within, IBD. In addition, a possible altered risk for developing IBD-drug related side-effects was explored.

Genomic DNA of 751 patients with IBD (209 patients with ulcerative colitis and 542 patients with Crohn's disease) and 930 healthy controls was genotyped for the *UGT1A1*28* promoter polymorphism, and genotype distribution was compared between patients and controls. Genotype phenotype interactions were also investigated.

Patients with Crohn's disease significantly less often bear the *UGT1A1*28* homozygous genotype compared to the control group, with an odds ratio of 0.64, 95% CI: 0.42 - 0.98. The ulcerative colitis group showed no significant differences compared to controls.

The homozygous state of the *UGT1A1*28* polymorphism, associated with higher serum bilirubin levels, may be protective for the development of Crohn's disease, suggesting that the anti-oxidant capacity of bilirubin may play a part.

Introduction

Over the last decades, extensive research has been performed to unravel the pathogenesis of inflammatory bowel disease (IBD), which encompasses both Crohn's disease and ulcerative colitis. It appears that both genetic and environmental factors, including the enteric microbiota and food antigens as well as an altered innate and adaptive immune system, all may be involved.[1] Oxidative stress is one of the pathways leading to cellular damage, which is also presumed to play an important role in the pathogenesis of IBD. Oxidative stress is the result of an imbalance between the production of reactive oxygen species (ROS), which are highly reactive due to the presence of unpaired electrons, and their removal by antioxidants.[2] Notably of importance in IBD is the production of large amounts of ROS, known as respiratory burst, by neutrophils and macrophages after activation by pro-inflammatory agents like cytokines, immune complexes or bacterial products.[3] In neutrophils derived from the intestinal mucosa of patients with IBD, an increased production of ROS has been observed.[4] Furthermore in patients with IBD, intestinal subepithelial myofibroblasts exhibited an increased oxidative state related to elevated levels of interleukin-6.[5] Oxidative stress might therefore be responsible for the increased synthesis of cytokines which accentuate and amplify the inflammatory state in patients with Crohn's disease.[5] A recent study characterized the ROS generated in immune peripheral cells in patients with Crohn's disease, and showed a significant increase in H_2O_2 in both active and remission phases of the disease, while anti-oxidative mechanisms catalyzing the decomposition of H_2O_2 were impaired.[6]

Intestinal damage in IBD has been suggested to be caused by an increased production of ROS and an impaired antioxidant capacity.[2,7,8] Bilirubin, which is formed during the catabolism of heme, has for a long time been regarded only as a toxic waste product, causing jaundice or kernicterus when not properly cleared. However, bilirubin has since proved to be a powerful endogenous chain-breaking antioxidant, and a protective role of bilirubin has been suggested in coronary artery disease, respiratory diseases and cancer.[9-14] A chronic mild form of unconjugated hyperbilirubinemia in the absence of liver disease or overt hemolysis is known as Gilbert's syndrome. In Caucasians, it is mainly caused by homozygosity for a polymorphism in the promoter region of the *UTG1A1* gene, known as the *UGT1A1*28* allele.[15,16] Functional analyses of the transcriptional promoter activity demonstrated that the *UGT1A1*28* allele reduces the transcription of the *UGT1A1* gene up to 20% of normal.[17] UGT1A1 is the only enzyme that catalyzes glucuronidation of bilirubin, which is the main determinant of elimination of bilirubin in humans.[18] Genotypes including the *UGT1A1*28* allele are associated with a reduced hepatic UGT1A1 enzyme activity up to 50%, with subsequent increased blood levels of unconjugated bilirubin, as compared with the most common *UGT1A1*1* genotypes.[15,19,20] Recent genome-wide association studies (GWAS) have also confirmed the substantial genetic influence of *UGT1A1* variants on bilirubin levels.[21,22]

In 2006, Seiderer and co-workers reported on a patient suffering from both Crohn's disease and Gilbert's syndrome, who developed nodular regenerative hyperplasia following treatment with azathioprine, suggesting a predisposition of the *UGT1A1*28* allele for abnormal thiopurine interactions.[23] In fact, the *UGT1A1*28* allele is strongly associated with irinotecan toxicity, a drug often used in the treatment of metastatic colorectal cancer.[24] We hypothesized that individuals bearing this allele, which is associated with reduced bilirubin conjugating enzyme activity and therefore increased bilirubin levels, are at a reduced risk for developing IBD. Since thiopurines (i.e. azathiopurine, 6-mercaptopurine and 6-thioguanine) are effective and widely used drugs in patients with IBD,[25], we also aimed to explore the risk of thiopurine-induced side effects in patients bearing this polymorphism in a nested case approach.

Materials and methods

Patients

Patients with a diagnosis of IBD based on accepted clinical, endoscopic, radiological and histological findings,[26] were recruited at the outpatient clinic of the Department of Gastroenterology, Radboud University Nijmegen Medical Center, being a tertiary referral center for IBD. A total number of 751 Caucasian patients (39% men, median age at diagnosis 26 years, range 4-76 years) were included, 542 patients with Crohn's disease (36% men, median age at diagnosis 24 years, range 4-76 years) and 209 patients with ulcerative colitis (48% men, median age at diagnosis 30 years, range 11-72 years). Furthermore, a total number of 930 healthy controls (43% men, median age 43 years, range 18-90 years), recruited from the referral region of our hospital, were included in the study. Blood samples were collected over a period of more than 10 years (1998-2010), while genotyping of all samples was performed in June 2010. Clinical characteristics of the patients, including disease extent according to the Montreal classification,[27] family history of IBD, necessity of surgery and occurrence of extra-intestinal manifestations, were obtained at last follow-up in 2010, as described before.[28] Basic characteristics of both cases and controls are given in supplementary table 5.1. Apart from clinical characteristics, we also collected data on thiopurine use and necessity to stop medication due to side effects. Side effects were categorized into gastro-intestinal intolerance (diarrhea, nausea and vomiting, abdominal pain), allergy (fever, skin rash, arthralgia/myalgia, Stevens Johnsons Syndrome), infection (Herpes zoster, fever of unknown origin, pneumonia), myelotoxicity (leukopenia and/or thrombocytopenia), hepatotoxicity (abnormal liver function tests) or pancreatic toxicity (abnormal pancreas enzymes, pancreatitis) and miscellaneous (fatigue, dizziness, generally unwell), based on laboratory results if appropriate. The mean follow-up of the patient cohort with Crohn's disease was 17.4 years (standard deviation (SD) 10.6), while the mean follow-up in the ulcerative colitis cohort was 15.1 years (SD 8.8).

Prior to study recruitment, 2 patients had been diagnosed with Gilbert's syndrome, 1 patient with ulcerative colitis and 1 patient with Crohn's disease.

Genotyping

DNA of both patients and controls was isolated from whole blood by use of the Pure Gene DNA isolation kit (Gentra Systems, Minneapolis, MN).[28] We determined the number of TA repeats in the promoter region of the *UGT1A1* gene by using the polymerase chain reaction (PCR) conditions and primers as described before by Monaghan *et al.*[16] Amplification was confirmed by agarose electrophoresis before fragments were resolved on 12% polyacrylamide gels (19:1 acrylamide/bisacrylamide; Biorad Laboratories, Veenendaal, The Netherlands) in Tris-borate-EDTA buffer. Electrophoresis was run at 400 V for 4 h and gels were stained with ethidium bromide for 15 min.[19] Fragments of 98 bp indicate the *UGT1A1*1* allele containing 6 TA repeats and fragments of 100 bp indicate the *UGT1A1*28* allele, containing 7 TA repeats.[29]

Statistical analysis

The observed genotype frequencies were tested for deviation from the Hardy-Weinberg equilibrium using Fisher's exact test. Differences in *UGT1A1* genotype distributions between patients and controls were determined by using the χ^2 test, and odds ratios (ORs) with 95% confidence interval (95% CI) were calculated for genotypes associated with predicted normal, versus predicted altered UGT1A1 enzyme activities (variant genotypes). In addition, we calculated the generalized odds ratio (OR_G), which utilizes the complete genotype distribution. It can be defined as the probability of a subject being more diseased relative to the probability of being less diseased, given that the more diseased subject has a higher mutational load.[30] The OR_G and 95% CI were calculated using the software "ORGGASMA" (downloaded from http://biomath.med.uth.gr). Lastly, we investigated whether an altered predicted enzyme activity influenced disease location or behavior, as well as for thiopurine side effects. Differences were calculated by using the χ^2 test or Fisher's exact test when appropriate. Statistical analysis was performed using GraphPad Prism version 4.00 (GraphPad Software, San Diego, CA).

Ethics

The ethical committee of region Nijmegen and Arnhem reviewed and approved the protocol under number CWOM-nr 9804-0100. Verbal informed consent was obtained from each patient before study participation in agreement with the approval and all samples were anonymized. Since research data were collected anonymously, at least verbal informed consent was needed according to national regulations. Therefore, no written informed consent procedure was introduced at time of data collection.

Results

Distribution of the UGT1A1 genotypes

Distribution of the *UGT1A1* genotypes in the control and patient groups, did fit the Hardy Weinberg equilibrium (p = 0.40 and p = 0.13, respectively). In patients with Crohn's disease, comparing the homozygous *UGT1A1*28* and the homozygous *UGT1A1*1* genotype, correlating with low and high bilirubin UGT enzyme activity respectively, revealed a significant difference (OR = 0.64, 95% CI 0.42-0.98; p = 0.04, table 5.1). For patients with ulcerative colitis however, such difference was not observed. No differences were seen when Crohn's disease patients with the heterogenous genotype, corresponding with predicted intermediate enzyme activity, were compared to the homozygous *UGT1A1*1* genotypes (table 5.1). Furthermore, there was a decreased incidence of extra-intestinal manifestations in *UGT1A1*28* allele barriers compared to wildtype carriers in patients with Crohn's disease (OR = 0.60, 95% CI 0.40-0.89; p = 0.009, table 5.2), which however lost significance after Bonferroni correction. The *UGT1A1*28* allele had no effect on disease location or the need of disease related surgery in patients with ulcerative colitis (table 5.3). The generalized odds ratio (OR_G), which provides an estimate of the magnitude of the association between disease status and genotype, was calculated by comparing patients with Crohn's disease and controls and showed a non significant association (OR_G = 0.83, 95% CI 0.69-1.01).

Possible relationship between UGT1A1 genotype and thiopurine-induced side effects

We subsequently explored the possible relationship between the *UGT1A1*28* allele and thiopurine induced side effects. Data on thiopurine use were available for 585 patients, 407 patients with Crohn's disease and 178 patients with ulcerative colitis. Of these patients, 343 patients used azathioprine, 53 patients used azathioprine in combination with 6-mercaptopurine (6-MP), 17 patients used azathioprine in combination with 6-thioguanine (6-TG), and 9 patients used azathioprine in combination with both 6-MP and 6-TG. A total of 126 thiopurine side effects were observed, 95 in patients with Crohn's disease and 31 in patients with ulcerative colitis. However, no significant association was observed between thiopurine related side effects and the presence of the *UGT1A1*28* allele (OR = 1.17, 95% CI 0.77-1.79; p = 0.46). However, when subgroups were analyzed, gastrointestinal intolerance to thiopurine therapy was associated with the *UGT1A1*28* allele (OR 2.26, 95% CI 1.11-4.62; p = 0.02, table 5.4). After Bonferroni correction however, this p-value did not remain significant.

Table 5.1 Distribution of the *UGT1A1* genotypes and corresponding ORs in patients with IBD versus controls

Genotype *UGT1A1*	All patients with IBD (n = 751)			Patients with CD (n = 542)			Patients with UC (n = 209)			Controls (n = 930)
	Number (%)	OR (95% CI)	p-value	Number (%)	OR (95% CI)	p-value	Number (%)	OR (95% CI)	p-value	Number (%)
**1/*1*	380 (50.6)	Reference		276 (50.9)	Reference		104 (49.8)	Reference		436 (46.9)
**1/*28*	319 (42.5)	0.89 (0.73-1.09)	0.28	232 (42.8)	0.89 (0.72-1.12)	0.34	87 (41.6)	0.89 (0.65-1.22)	0.47	410 (44.1)
**28/*28*	52 (6.9)	0.71 (0.49-1.03)	0.08	34 (6.3)	0.64 (0.42-0.98)	0.04	18 (8.6)	0.90 (0.52-1.56)	0.78	84 (9.0)

OR, Odds ratio; CI, confidence interval; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease

Table 5.2 *UGT1A1* genotype-phenotype correlations in patients with Crohn's disease[#]

	**1/*1* (n = 268)	**1/*28* (n = 223)	**28/*28* (n = 27)	Odds ratio (95% CI)	p-value
Disease location[†]					
Ileal	89	83	9	Reference	
Colonic	71	46	3	0.67 (0.42-1.06)	0.09
Ileocolonic	102	88	15	0.98 (0.65-1.46)	0.91
Isolated upper disease[+]	15	15	4	1.23 (0.59-2.56)	0.59
Disease behaviour[†]					
Non stricturing/ penetrating	85	69	9	Reference	
Stricturing	45	42	0	1.02 (0.60-1.71)	0.95
Penetrating	132	106	18	1.02 (0.69-1.52)	0.91
Age at diagnosis[†]					
< 16 years	24	28	1	Reference	
Between 17 and 40 years	208	171	23	0.77 (0.43-1.37)	0.38
Above 40 years	36	21	7	0.64 (0.31-1.34)	0.24
Surgery	164	132	19	0.97 (0.68-1.38)	0.85
Extra-intestinal	92/246	54/201	6/26	0.60 (0.40-0.89)	0.01
Family history of IBD	38/132	31/108	4/20	0.93 (0.54-1.60)	0.80

[#]The *UGT1A1*1/*1* genotypes were compared with the patients bearing the *UGT1A1*28* allele (both homo- and heterozygous). Note that phenotypes of not all patients were available. Significant p-values are given in bold letters including corresponding odds ratios (OR) and 95% confidence intervals (CI). However, none of these P-values remained significant after Bonferroni correction ($p > 0.05$).
[+]Patients were classified as having disease localization in the upper gastrointestinal tract next to ileal, ileo-colonic or colonic localization
[†]According to the Montreal Classification

Table 5.3 *UGT1A1* genotype-phenotype correlations in patients with ulcerative colitis[#]

	**1/*1*	**1/*28*	**28/*28*	Odds ratio (95% CI)	p-value
Disease localization (n = 176)[†]					
Proctitis	5	4	2	Reference	
Left sided	25	29	6	1.17 (0.32-4.25)	0.82
Pancolitis	57	39	9	0.70 (0.20-2.44)	0.58
Surgery (n = 194)	30/96	23/80	4/18	0.84 (0.45-1.55)	0.58

[#]The *UGT1A1*1/*1* genotypes were compared with the patients bearing the *UGT1A1*28* variant (both homozygous and heterozygous). Note that phenotypes of not all patients were available
[†]According to the Montreal Classification.

Table 5.4 Thiopurine side effects stratified by *UGT1A1* genotype[#]

	**1/*1* (n = 208)	**1/*28* (n = 184)	**28/*28* (n = 32)	Odds ratio (95% CI)	p-value
No adverse event	143	126	18	Reference	
Total adverse events	65	58	14	1.10 (0.73-1.65)	0.65
Gastrointestinal intolerance	12	24	2	**2.15 (1.05-4.43)**	**0.03**
Allergy	24	17	5	0.91 (0.49-1.70)	0.77
Infection	3	0	1	0.33 (0.03-3.22)	0.62
Myelotoxicity	9	6	1	0.77 (0.28-2.13)	0.62
Hepatotoxicity	8	4	3	0.87 (0.31-2.46)	0.79
Pancreas toxicity	5	6	0	1.19 (0.36-3.99)	1.00
Miscellaneous	4	1	2	0.74 (0.16-3.39)	1.00

[#]The *UGT1A1*1/*1* genotypes were compared with the patients bearing the *UGT1A1*28* allele (both homo- and heterozygous).

Discussion

In our cohort, homozygosity for the *UGT1A1*28* allele, which correlates with low enzyme activity, exerted a protective effect against Crohn's disease. The 9.0% prevalence of homozygosity for the *UGT1A1*28* allele in our control population is lower compared to homozygosity rates of 12% reported in other European studies,[16, 31] which might even more emphasize the significance of our finding. A detailed genotype-phenotype analysis revealed a weak association of the *UGT1A1*28* allele with a decreased incidence of extra-intestinal manifestations, which however did not remain significant after Bonferroni correction. The results of our study could imply that an increased blood level of unconjugated bilirubin confers a protective effect on the development of Crohn's disease. In accordance with this hypothesis, Koutroubakis and co-workers showed that patients with inflammatory bowel disease had a decreased serum antioxidant capacity as compared to healthy controls, with bilirubin acting as one of the major endogenous anti-oxidants accounting for this antioxidant capacity.[32] Furthermore, several animal studies have indicated a protective role of the products formed during heme catabolism, including heme oxygenase (HO), carbon monoxide (CO) and biliverdin-bilirubin, in the pathogenesis of inflammatory bowel disease.[33-35] Decreased levels of bilirubin have also been associated with other auto-inflammatory diseases in which oxidative stress also seems to be pathogenically involved, including rheumatoid arthritis and systemic lupus erythematosus.[36-39]

In the catabolism of heme, the stress responsive enzyme HO-1 is involved, which together with HO-2, are the rate limiting enzymes in the formation of biliverdin, which successively is converted into bilirubin, free iron and CO.[40] In an experimental colitis model administering dextran sodium sulfate (DSS) to rats, Berberat et al. showed that induction of intestinal HO-1 ameliorates manifestations of DSS induced

induced colitis. Daily intraperitoneal injections of biliverdin resulted in a comparable protective effect, indicating that the generation of bilirubin by the up-regulation of HO-1 may be the key mediator in suppressing inflammatory damage in acute colitis.[41] In rats treated with trinitrobenzene sulphonic acid (TNBS) to induce colitis, increased expression and activity of HO-1, as measured by formation of bilirubin, was observed after TNBS challenge.[42]

In this study we explored the possible link between Gilbert's syndrome and the development of thiopurine-induced side effects, by investigation the association of the *UGT1A1*28* allele and thiopurine related side effects.[23] No association was found; while a sub analysis revealed that bearing the *UGT1A1*28* allele was associated with gastrointestinal intolerance for thiopurines. This association however, did not remain significant after correction for multiple comparisons. Furthermore, this finding might also be explained as a by chance finding due to the small groups analyzed. Thiopurine prodrugs are converted intracellularly into cytotoxic 6-thioguanine nucleotides (6-TGNs) to exert their therapeutic effect, but UGTs seem to be not involved in the metabolism of the prodrugs. Therefore it is unclear how UGT1A1 might be involved in the occurrence of gastrointestinal intolerance. An enzyme which certainly is involved in the catabolism of thiopurines, the thiopurine S methyltransferase (TPMT), is a major determinant for 6-TGNs levels.[43] High levels of 6-TGNs are associated with thiopurine side effects and different variants of TPMT exist which may result in altered TPMT activity. A recent meta-analysis showed an association between TPMT polymorphisms and thiopurine side effects in patients with IBD.[44] However, not all side effects of thiopurine therapy could be explained by TPMT polymorphisms, which implies that other mechanisms, unrelated to TPMT, might play a role.[45] A limitation of our study might be that we did not measure blood, serum or plasma levels of bilirubin in our patients, but only used the *UGT1A1*28* allele as an indirect parameter for hyperbilirubinemia. However, multiple studies have confirmed the relationship between the *UGT1A1*28* polymorphism and elevated plasma/serum/blood levels of bilirubin.[16, 18, 46]

Conclusion

Homozygosity for the *UGT1A1*28* allele exerted a protective effect against the development of Crohn's disease, as well on the development of extra-intestinal manifestations of Crohn's disease in a Dutch cohort of IBD patients. Most likely this protective effect is due to the antioxidant capacity of the slightly elevated bilirubin levels. If firmly established, this might possibly lead to new strategies in the prevention or support treatment of Crohn's disease by supplementation with antioxidants.

References

1. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448:427-434.
2. Rezaie A, Parker RD, Abdollahi M. Oxidative Stress and Pathogenesis of Inflammatory Bowel Disease: An Epiphenomenon or the Cause? Dig Dis Sci 2007;52:2015-2021.
3. Kruidenier L, Verspaget HW. Review article: oxidative stress as a pathogenic factor in inflammatory bowel disease — radicals or ridiculous? Aliment Pharmacol Ther 2002;16:1997-2015.
4. Simmonds NJ, Allen RE, Stevens TR, et al. Chemiluminescence assay of mucosal reactive oxygen metabolites in inflammatory bowel disease. Gastroenterology 1992;103:186-196.
5. Catarzi S, Favilli F, Romagnoli C, et al. Oxidative state and IL-6 production in intestinal myofibroblasts of Crohn's disease patients. Inflamm Bowel Dis 2011;17:1674-1684.
6. Beltrán B, Nos P, Dasí F, et al. Mitochondrial dysfunction, persistent oxidative damage, and catalase inhibition in immune cells of naïve and treated Crohn's disease. Inflamm Bowel Dis 2010;16:76-86.
7. Torres MI, Ríos A. Current view of the immunopathogenesis in inflammatory bowel disease and its implications for therapy. World J Gastroenterol 2008;14:1972-1980.
8. Iborra M, Moret I, Rausell F, et al. Role of oxidative stress and antioxidant enzymes in Crohn's disease. Biochem Soc Trans 2011;39:1102-1106.
9. Stocker R, Yamamoto Y, McDonagh AF, et al. Bilirubin Is an Antioxidant of Possible Physiological Importance. Science 1987; 235:1043-1046.
10. Stocker R, Glazer AN, Ames BN. Antioxidant activity of albumin-bound bilirubin. Proc Natl Acad Sci U S A 1987;84:5918-5922.
11. Mayer M. Association of Serum Bilirubin Concentration with Risk of Coronary Artery Disease. Clinical Chemistry 2000;46:1723-1727.
12. Horsfall LJ, Rait G, Walters K, et al. Serum bilirubin and risk of respiratory disease and death. JAMA 2011;305:691-697.
13. Zucker SD, Horn PS, Sherman KE. Serum bilirubin levels in the U.S. population: gender effect and inverse correlation with colorectal cancer. Hepatology 2004;40:827-835.
14. Temme EH, Zhang J, Schouten EG, et al. Serum bilirubin and 10-year mortality risk in a Belgian population. Cancer Causes Control 2001;12:887-894.
15. Bosma PJ, Chowdhury JR, Bakker C, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. N Engl J Med 1995;333:1171-1175.
16. Monaghan G, Ryan M, Seddon R, et al. Genetic variation in bilirubin UPD glucuronosyltransferase gene promoter and Gilbert's syndrome. Lancet 1996;347:578-581.
17. Bosma PJ. Inherited disorders of bilirubin metabolism. J Hepatol 2003;38:107-117.
18. Chen YH, Hung SC, Tarng DC. Serum Bilirubin Links UGT1A1*28 Polymorphism and Predicts Long-Term Cardiovascular Events and Mortality in Chronic Hemodialysis Patients. Clin J Am Soc Nephrol 2011;6:567-574.
19. Raijmakers MTM, Jansen PLM, Steegers EAP, et al. Association of human liver bilirubin UDP-glucuronosyltransferase activity with a polymorphism in the promoter region of the UGT1A1 gene. J Hepatol 2000;33:348–351.
20. Peters WHM, te Morsche RHM, Roelofs HMJ. Combined polymorphisms in UDP glucuronosyltransferases 1A1 and 1A6: implications for patients with Gilbert's syndrome. J Hepatol 2003;38:3-8.
21. Johnson AD, Kavousi M, Smith AV, et al. Genome-wide association meta-analysis for total serum bilirubin levels. Hum Mol Genet 2009;18:2700-2710.

22. Bielinski SJ, Chai HS, Pathak J, et al. Mayo Genome Consortia: a genotype-phenotype resource for genome-wide association studies with an application to the analysis of circulating bilirubin levels. Mayo Clin Proc 2011;86:606-614.
23. Seiderer J, Zech CJ, Diebold J, et al. Nodular regenerative hyperplasia: a reversible entity associated with azathioprine therapy. Eur J Gastroenterol Hepatol 2006;18:553-555.
24. Lee S, Mcleod HL. Pharmacogenetic tests in cancer chemotherapy: what physicians should know for clinical application. J Pathol 2011;223:15-27.
25. Burger D, Travis SPL. Conventional Medical Management of Inflammatory Bowel Disease. Gastroenterology 2011;140:1827-1837.
26. Podolsky DK. Inflammatory bowel disease. New Engl J Med 2002;347:417-429.
27. Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol 2005;19:5A-36A.
28. de Vries HS, te Morsche RHM, van Oijen MGH, et al. The Functional 765G→C Polymorphism of the COX-2 Gene May Reduce the Risk of Developing Crohn's Disease. PLoS One 2010;5:e15011.
29. Lacko M, Roelofs HMJ, te Morsche RHM, et al. Genetic polymorphism in the conjugating enzyme UGT1A1 and the risk of head and neck cancer. Int J Cancer 2010;127:2815–2821.
30. Zintzaras E. The generalized odds ratio as a measure of genetic risk effect in the analysis and meta-analysis of association studies. Stat Appl Genet Mol Biol. 2010;9:Article21.
31. Rauchschwalbe SK, Zühlsdorf MT, Schühly U, et al. Predicting the risk of sporadic elevated bilirubin levels and diagnosing Gilbert's syndrome by genotyping UGT1A1*28 promoter polymorphism. Int J Clin Pharmacol Ther 2002;40:233-240.
32. Koutroubakis IE, Malliaraki N, Dimoulios PD, et al. Decreased total and corrected antioxidant capacity in patients with inflammatory bowel disease. Dig Dis Sci 2004;49:1433-1437.
33. Naito Y, Takagi T, Yoshikawa T. Heme oxygenase-1: a new therapeutic target for inflammatory bowel disease. Aliment Pharmacol Ther 2004;20(Suppl. 1):177–184.
34. Sheikh SZ, Hegazi RA, Kobayashi T, et al. An anti-inflammatory role for carbon monoxide and heme oxygenase-1 in chronic th2-mediated murine colitis. J Immunol. 2011;186:5506-13.
35. Hegazi RA, Rao KN, Mayle A, et al. Carbon monoxide ameliorates chronic murine colitis through a heme oxygenase 1-dependent pathway. J Exp Med. 2005;202:1703-13.
36. Lozovoy M, Simão A, Panis C, et al. Oxidative stress is associated with liver damage, inflammatory status, and corticosteroid therapy in patients with systemic lupus erythematosus. Lupus. 2011;20:1250-1259.
37. Wruck CJ, Fragoulis A, Gurzynski A, et al. Role of oxidative stress in rheumatoid arthritis: insights from the Nrf2-knockout mice. Ann Rheum Dis 2011;70:844-850.
38. Fischman D, Valluri A, Gorrepati VS, et al. Bilirubin as a Protective Factor for Rheumatoid Arthritis: An NHANES Study of 2003 - 2006 Data. J Clin Med Res 2010;2:256-260.
39. Vítek L, Muchová L, Jancová E, et al. Association of systemic lupus erythematosus with low serum bilirubin levels. Scand J Rheumatol 2010 ;39:480-484.
40. Motterlini R, Otterbein LE. The therapeutic potential of carbon monoxide. Nature Rev Drug Disc 2010;9:728-743.
41. Berberat PO, A-Rahim YI, Yamashita K, et al. Heme oxygenase-1-generated biliverdin ameliorates experimental murine colitis. Inflamm Bowel Dis 2005;11:350-359.

42. Varga C, Laszlo F, Fritz P, et al. Modulation by heme and zinc protoporphyrin of colonic heme oxygenase-1 and experimental inflammatory bowel disease in the rat. Eur J Pharmacol 2007;561:164-171.
43. Ford LT, Berg JD. Thiopurine S-methyltransferase (TPMT) assessment prior to starting thiopurine drug treatment; a pharmacogenomic test whose time has come. J Clin Pathol 2010; 63:288-95.
44. Dong XW, Zheng Q, Zhu MM, et al. Thiopurine S-methyltransferase polymorphisms and thiopurine toxicity in treatment of inflammatory bowel disease. World J Gastroenterol 2010; 16:3187-3195.
45. Bourgine J, Garat A, Allorge D, et al. Evidence for a functional genetic polymorphism of the Rho-GTPase Rac1. Implication in azathioprine response? Pharmacogenet Genomics 2011;21:313-324.
46. Borucki K, Weikert C, Fisher E, et al. Haplotypes in the UGT1A1 gene and their role as genetic determinants of bilirubin concentration in healthy German volunteers. Clin Biochem 2009;42:1635-1641.

Supplementary table 5.1 Basic characteristics of patients and controls

	Controls (n=930)		Crohn's disease (n=542)		Ulcerative colitis (n=209)	
Median age, years (range)	43 (18-90)		44 (17-84)		45 (19-82)	
Sex						
Male	396	43%	200	37%	97	46%
Female	534	57%	342	63%	112	54%

Chapter 6

Genetic association analysis of the functional c.714T>G polymorphism and mucosal expression of dectin-1 in inflammatory bowel disease

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Dectin-1 is a pattern recognition receptor (PRR) expressed by myeloid cells that specifically recognizes β-1,3 glucan, a polysaccharide and major component of the fungal cell wall. Upon activation, dectin-1 signaling converges, similar to NOD2, on the adaptor molecule CARD9 which is associated with inflammatory bowel disease (IBD). An early stop codon polymorphism (c.714T>G) in *DECTIN-1* results in a loss-of-function (p.Y238X) and impaired cytokine responses, including TNF-α, interleukin (IL)-1β and IL-17 upon in vitro stimulation with Candida albicans or β-glucan. The aim of the present study was to test the hypothesis that the *DECTIN-1* c.714T>G (p.Y238X) polymorphism is associated with lower disease susceptibility or severity in IBD and to investigate the level of dectin-1 expression in inflamed and non-inflamed colon tissue of IBD patients.

Paraffin embedded tissue samples from non-inflamed and inflamed colon of IBD patients and from diverticulitis patients were immunohistochemically stained for dectin-1 and related to CD68 macrophage staining. Genomic DNA of IBD patients (778 patients with Crohn's disease and 759 patients with ulcerative colitis) and healthy controls (n = 772) was genotyped for the c.714T>G polymorphism and genotype-phenotype interactions were investigated.

Increased expression of dectin-1 was observed in actively inflamed colon tissue, as compared to non-inflamed tissue of the same patients. Also an increase in dectin-1 expression was apparent in diverticulitis tissue. No statistically significant difference in *DECTIN-1* c.714T>G allele frequencies was observed between IBD patients and healthy controls. Furthermore, no differences in clinical characteristics could be observed related to *DECTIN-1* genotype, neither alone, nor stratified for *NOD2* genotype.

Our data demonstrate that dectin-1 expression is elevated on macrophages, neutrophils, and other immune cells involved in the inflammatory reaction in IBD. The *DECTIN-1* c.714T>G polymorphism however, is not a major susceptibility factor for developing IBD.

Introduction

Inflammatory bowel disease (IBD), is an idiopathic, chronic, relapsing inflammatory disorder of the gastrointestinal tract. It is commonly accepted that IBD is caused by an exaggerated cell mediated immune response to intestinal microbiota in genetically susceptible individuals.[1, 2] IBD mainly involves two distinct diseases, which show some overlap: Crohn's disease (CD) and ulcerative colitis (UC). Genetic susceptibility is more pronounced in CD compared to UC.[3] Several susceptibility loci for developing CD have been identified in the past decades including the NOD2 gene within the *IBD1* locus.[4] The established association of *NOD2* (*CARD15*) with CD emphasizes the important role of the intestinal microbiota in the pathogenesis of CD, since NOD2 acts as an intracellular pattern recognition receptor (PRR) recognizing bacterial peptidoglycans.[5, 6]

Dectin-1 (*CLEC7A*) is a pattern-recognition receptor expressed by myeloid cells which specifically recognizes β-(1,3)-glucan, a polysaccharide and component of the fungal cell wall. As a result, dectin-1 is involved in recognition of fungi such as *Candida albicans* and *Aspergillus fumigatus*. Upon activation, dectin-1 recruits spleen tyrosine kinase (Syk) which in turn activates NF-κB, requiring the adaptor molecule Caspase Activating Recruitment Domain 9 (CARD9), a key adaptor for non-Toll Like Receptor (TLR) signal transduction.[7] Although not the exclusive pathway, CARD9 also has a critical function in NOD2-mediated activation of the kinases p38 and Jnk, required for the production of pro-inflammatory cytokines in innate immune responses to intracellular pathogens.[8] LeibundGut-Landmann *et al.* showed that dectin-1-Syk-CARD9 signaling induces dendritic cell (DC) maturation and secretion of pro-inflammatory cytokines like interleukin (IL)-6, TNF-α, IL-17 and IL-23.[9] Furthermore, Zhernakova *et al.* identified *CARD9* as a susceptibility locus for IBD.[10] Recently, the *DECTIN-1* polymorphism c.714T>G on chromosome 12p13 has been described, with a transition from a tyrosine to an early stop codon on amino acid position 238 (p.Y238X).[11] The functional consequence of this polymorphism is a complete loss-of-function, and immune cells expressing this truncated protein produce significantly less cytokines, including TNF-α, IL-1β and IL-17, upon *in vitro* stimulation with β-glucan or *Candida albicans*.[12]

Th17 responses are considered to be involved in the pathogenesis of auto-immune diseases. This T cell subset appears to play a role in the etiology of CD since IL-17 is up-regulated in the intestine of IBD patients.[13] Interestingly, both NOD2 and dectin-1 are shown to be capable of inducing Th17 responses after activation.[9, 14] In this respect, the *DECTIN-1* c.714T>G polymorphism could influence the Th17 response towards fungi such as Candida albicans, a commensal microorganism of the gastrointestinal tract. *Candida albicans* is also one of the immunogens for developing antibodies against *Saccharomyces cerevisiae* (ASCA), which are regularly observed in patients with CD.[15, 16]

Taking together the data from various studies, a dysregulation of the immune response to the commensal Candida albicans through dectin-1 and IL-17 release might play a role in the pathogenesis of CD.

As stated above, activation of NOD2 and dectin-1 leads to signaling through a shared pathway (CARD9). The importance of this pathway in CD is demonstrated by the fact that mutations in *NOD2* and *CARD9* and the presence of circulating ASCA, are associated with CD. Since the c.714T>G polymorphism within *DECTIN-1* results in a loss of function, we hypothesized that this polymorphism could be potentially protective against developing IBD. Therefore, we aimed to elucidate the role of the *DECTIN-1* c.714T>G (p.Tyr238X) polymorphism in patients with IBD, focusing on the occurrence and the clinical severity of IBD.

Methods

Patients

Patients with a diagnosis of IBD were recruited from the outpatient clinics of three university hospitals in the Netherlands: Radboud University Nijmegen Medical Centre (CD: n = 161, UC: n = 212), VU University Medical Centre Amsterdam (CD: n = 177, UC: n = 148) and the University Medical Centre Groningen (CD: n = 308, UC: n = 214), and one regional hospital: St. Anna Hospital, Geldrop (CD: n = 132, UC: n = 185). Healthy controls were recruited at the Radboud University Nijmegen Medical Centre and at the University Medical Centre Groningen (n = 772).

Diagnosis of IBD was based on accepted clinical, endoscopic, radiological and histological findings.[17] Clinical data on patients were retrieved by retrospective collection from patients' clinical charts. Clinical data on patients from the VU University Medical Centre were collected prospectively. The following data were obtained from patients with CD: age, age at diagnosis, gender, familial or sporadic IBD, disease localization and behavior of disease (according to the Vienna classification[18]), extra-intestinal manifestations, peri-anal disease, and surgery for CD. For patients with UC, the following data were obtained: age, age at diagnosis, disease location (according to the Montreal classification[19]), familial or sporadic IBD, extra intestinal manifestations, surgery for UC, and occurrence of colorectal cancer.

The ethical committee of region Nijmegen and Arnhem reviewed and approved the protocol under number CWOM-nr 9804-0100. Verbal informed consent was obtained from each patient before study participation in agreement with the approval and all samples were anonymized. Given the fact that all research data were anonymously collected, at least verbal informed consent was needed according to national regulations. Therefore, since written informed consent was not required, no written informed consent procedure was introduced at time of data collection.

Genotyping of c.714T>G polymorphism in DECTIN-1

Genomic DNA was isolated from peripheral venous blood using standard techniques and stored at 4°C. Genotyping of the c.714T>G (p.Y238X) polymorphism in exon 6 of the *DECTIN-1* gene in the patient and healthy control groups from Nijmegen, Amsterdam and Geldrop was performed by applying the predesigned TaqMan SNP assay C_33748481_10 (rs 16910526) on the 7300 ABI Real-Time PCR system (both from Applied Biosystems, Foster City, CA, USA) using 96-well plates.

Genotyping of the IBD cohort and healthy controls from Groningen, was performed at the Department of Genetics, UMC Groningen, the Netherlands, applying the same predesigned TaqMan SNP assay, using the 7900 ABI Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The patient and control DNA samples from Groningen were processed in 384-well plates and each plate also contained 16 genotyping controls (4 duplicates of the Centre d'Etudes du Polymorphisme Humain (CEPH) DNA samples 123002,102405,090203 and 081505). For all polymorphisms we obtained >99.8% concordance between our CEPH genotype data and the CEU (European ancestry) data available from HapMap.

Genotyping of NOD2 variants

Data on the three common *NOD2* variants were available for CD patients from Groningen and Nijmegen (p.Arg702Trp; n =437, p.Gly908Arg; n= 446, p.Leu1007ProfsX2; n= 436). Genotyping of these three NOD2 variants (c.2104C.T (p.Arg702Trp), c.2722G.C (p.Gly908Arg), c.3019_3020insC (p.Leu1007ProfsX2)) has been described before.[20]

Immunohistochemical staining

Dectin-1 and CD68 protein expression were evaluated by immunohistochemical staining in paraffin embedded normal and inflamed colon tissue of five IBD patients and 4 patients with diverticulitis, all homozygous for the *DECTIN-1* wild-type allele (T/T). The applied primary antibodies were a monoclonal mouse-anti-human dectin-1 antibody (MAB 1859, purchased from R&D Biosystems, Minneapolis, MN, USA), used in a concentration of 5 μg/ml and a monoclonal mouse-anti-human antibody against CD68 (MCA1815T, purchased from AbD Serotec, Oxford, UK) which was diluted 100 times before use. After overnight incubation with the primary antibody, the tissue sections were incubated for 1 hour with a secondary antibody after washing with PBS. Subsequently the staining was visualized by applying ABC complex and DAB solution. Sections were counterstained with haematoxylin.

Statistics

Statistical analysis was performed by using SPSS statistical software, version 16.0 (SPSS Inc., Chicago, IL). Controls and IBD patients were tested for Hardy Weinberg equilibrium. Allele frequencies were compared between patients and controls using the χ^2 test. P-values were obtained by comparing individuals carrying at least one *DECTIN-1* G allele (G/G genotype and T/G genotype) with wild-type individuals (T/T genotype). Continuous variables were compared using Student t-tests. Strength of association between genotype and phenotype is given as odds ratio with 95% confidence interval (CI). Statistical interaction between *NOD2* variants and the c.714T>G polymorphism regarding clinical characteristics, was investigated by comparing patients carrying a *NOD2* susceptibility allele in combination with carrying one or two copies of the *DECTIN-1* G allele, to patients not bearing any of these *NOD2* or *DECTIN-1* minor alleles.

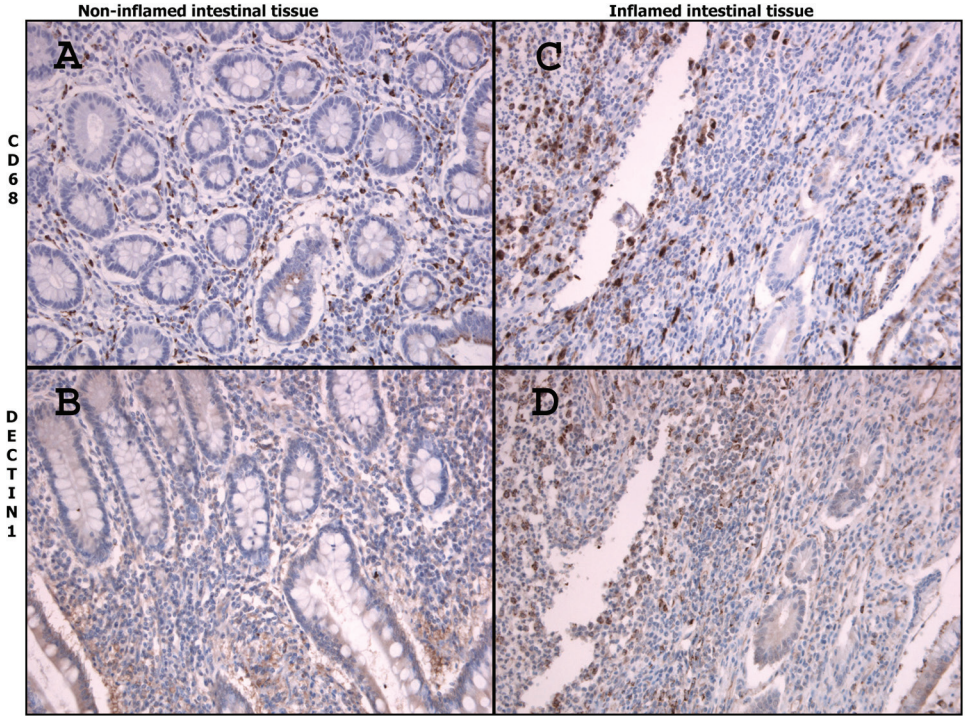
This combined analysis for c.714T>G and *NOD2* was performed for each of the three *NOD2* susceptibility alleles. A P-value <0.05 was considered significant.

Results

Protein expression of dectin-1 in intestinal tissue

Dectin-1 and CD68 staining was performed on matched intestinal tissue samples from five IBD patients, either inflamed or non-inflamed, as depicted in figure 6.1 and 6.2. Dectin-1 expression is mainly present on macrophages as showed by staining for CD68 (figure 6.1). Furthermore, dectin-1 also appears to be weakly expressed on neutrophils, the membrane of endothelial and epithelial cells and in the submucosal neuronal plexus of Meissner (not shown). Dectin-1 appeared to be up-regulated within inflamed colon tissue due to increased expression of dectin-1 on inflammatory cells and increased influx of inflammatory cells (figure 6.1).

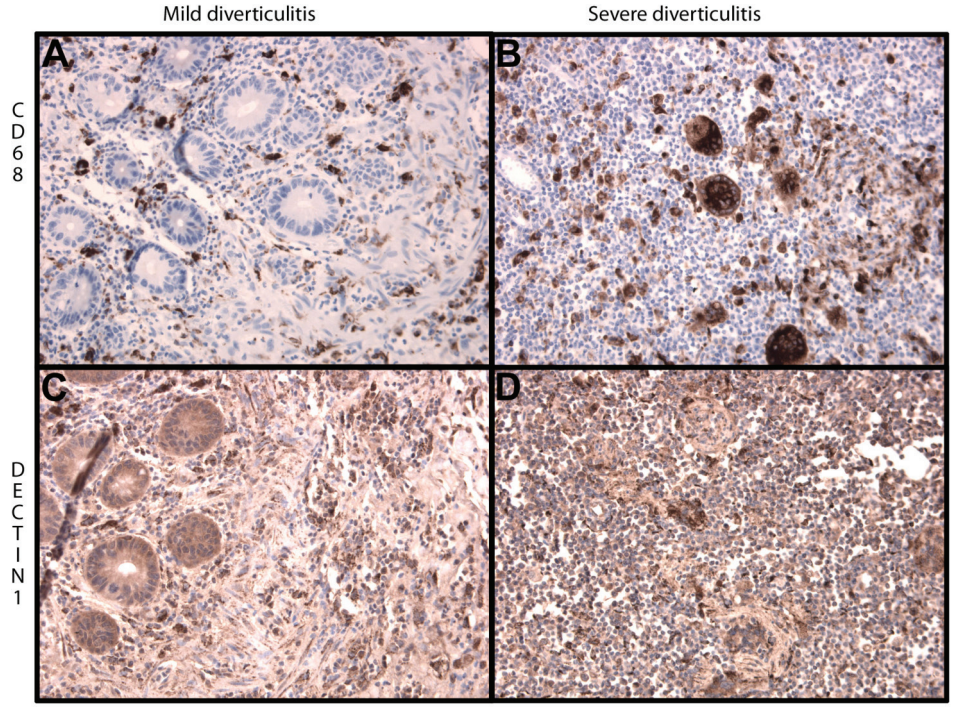
Figure 6.1 Representative immunohistochemical staining of DECTIN-1 and CD-68 in inflamed and non-inflamed intestine of the same specimen in Crohn's disease (250× magnified).



Macrophages are present in non-inflamed intestinal tissue but are present in increased numbers in inflamed tissue (pictures A and C). The expression of DECTIN-1 is increased in inflamed intestinal tissue compared to non-inflamed intestinal tissue (pictures B and D).

In order to test whether this increased expression of dectin-1 is IBD specific, additional staining was performed on matched intestinal tissue samples from 4 patients with diverticulitis. As shown in figure 6.2, increased expression of dectin-1 was also observed in patients with severe diverticulitis compared to mild diverticulitis. As is true for patients with IBD, expression of dectin-1 is mainly present on macrophages as shown by CD68 staining.

Figure 6.2 Representative immunohistochemical staining of dectin-1 and CD68 in mild and severe diverticulitis (250× magnified).



Macrophages are present in intestinal tissue of mild diverticulitis but are present in increased numbers in severe diverticulitis (pictures A and B). Also, the expression of dectin-1 is increased in severe diverticulitis compared to mild diverticulitis (pictures C and D).

Distribution of the DECTIN-1 c.714T>G polymorphism in IBD patients and healthy controls

Characteristics of the study population and healthy controls are depicted in table 6.1. Genotype frequencies of healthy controls were in Hardy Weinberg equilibrium. Carriage of at least one copy of the G allele of the *DECTIN-1* polymorphism was 14% in the CD, 14% in the UC and 17% in the healthy control group. The frequency of the G allele was 9.8% in the healthy controls, 8.1% in CD patients and 7.7% in UC patients. Overall, no significant differences were observed between patients with IBD and healthy controls regarding allele frequencies of the *DECTIN-1* polymorphism.

Table 6.1 Distribution of genotypes of wild-type, heterozygous and homozygous individuals for the c.714T>G polymorphism

DECTIN-1 status	Controls	%	Crohn's disease	%	p-value	Ulcerative colitis	%	p-value
Total cohort, number	772	100	778	100		759	100	
T/T	642	83.2	667	85.7	0.16	655	86.3	0.09
T/G	122	15.8	106	13.6		100	13.2	
G/G	8	1.0	5	0.6		4	0.5	

Correlation of the DECTIN-1 c.714T>G polymorphism with clinical characteristics of IBD patients

Patients with CD carrying one or two copies of the *DECTIN-1* G allele were compared to patients with the wild-type genotype T/T with regard to age at diagnosis, gender, family history of IBD, localization of disease and disease behavior, extra intestinal and peri-anal disease and surgery related to CD (table 6.2). Patients with UC were likewise compared according to the *DECTIN-1* genotypes regarding age at diagnosis, gender, localization of disease, extra-intestinal disease, development of malignancies, surgery related to UC and a positive family history for IBD (table 6.3). No statistical significant associations were observed between the c.714T>G polymorphism and specific phenotypes.

The DECTIN-1 c.714T>G polymorphism stratified by NOD2 status and clinical characteristics of IBD patients

CD patients carrying one or two copies of the G allele of the *DECTIN-1* gene were stratified by *NOD2* status. A *NOD2* risk genotype was defined as carrying at least one of the three common *NOD2* disease susceptibility alleles (c.2104C>T (p.Arg702Trp), c.2722G>C (p.Gly908Arg), c.3019_3020insC (p.Leu1007fsX1008)). Combinations of NOD2 risk carriers and *DECTIN-1* c.714T>G carriers were compared to patients not bearing any of these *NOD2* or *DECTIN-1* minor alleles, regarding clinical characteristics. No statistical significant interaction between *DECTIN-1* c.714T>G and one of the *NOD2* variants was observed (data not shown).

Discussion

Signaling through dectin-1, known for its recognition of the fungal component β-glucan, has been described to be involved in several immunological pathways. Dectin-1 amplifies pro-inflammatory cytokine production induced by TLR2 and TLR4, and primes Th1, Th17 and cytotoxic T cell responses induced by dendritic cells.[9, 21] The *DECTIN-1* c.714T>G polymorphism results in a loss-of-function of dectin-1, and we hypothesized that this polymorphism could be potentially protective in either the susceptibility to or the disease severity of IBD.

Table 6.2 Association between DECTIN-1 genotypes and clinical characteristics in a subset of Crohn's disease patients from whom detailed phenotypic data were available (N = 778)

Characteristic	Total cohort CD	(%)	T/T	(%)	T/G	(%)	G/G	(%)	Odds ratio*	95% CI*	
Mean age at diagnosis, yr (SD)	29.6 (±12.4)		29.6 (±12.3)		29.4 (±12.6)		30.8 (±17.3)				
Male gender	261	(33.5)	222/ 667	(33.3)	37/ 106	(34.9)	2/ 5	(40.0)			
Familial IBD (N = 631)	131	(20.8)	107/ 588	(20.3)	24/ 101	(24.0)	0/ 5	(0)	1.32	0.78	2.17
Localization (Vienna Classification) (n=778)											
L1: ileum	196		164/ 667	(24.6)	32/ 106	(30.2)	0/ 0	(0)	1.24	0.80	1.94
L2: colon	194		163	(24.2)	28	(26.4)	3	(60.0)	1.20	0.76	1.88
L3: ileocolonic	388		340	(51.0)	46	(43.4)	2	(40.0)	0.73	0.49	1.10
L4: upper disease	43		36	(5.4)	7	(6.6)	0	(0)	1.18	0.51	2.72
Disease behavior (Montreal classification) (n=776)											
B1: non structuring, non penetrating	291	(37.5)	250/ 665	(37.6)	39/ 106	(36.8)	2/ 5	(40.0)	0.97	0.64	1.47
B2: structuring	215	(27.7)	187	(28.1)	27	(25.5)	1	(20.0)	0.86	0.54	1.37
B3: penetrating	270	(34.8)	228	(34.3)	40	(37.7)	2	(40.0)	1.16	0.77	1.77
Extraintestinal disease (n=750)	151	(20.1)	125/ 642	(19.5)	26/ 103	(25.2)	0/ 5	(0)	1.31	0.81	2.13
Perianal disease (n=643)	177	(27.5)	149/ 548	(27.2)	26/ 90	(28.9)	2/5	(40.0)	1.12	0.69	1.81
Surgery (n=774)	411	(53.1)	355/ 664	(53.5)	54/ 105	(51.4)	2/ 5	(40.0)	0.90	0.60	1.35

*Carriers of the mutant allele (T/G and G/G) were compared to wild-types (T/T).
Values are presented as absolute numbers (percentages).

Table 6.3 Association between DECTIN-1 genotypes and clinical characteristics in a subset of ulcerative colitis patients from whom detailed phenotypic data were available (N =759)

Characteristic	Total cohort UC	(%)	T/T	(%)	T/G	(%)	G/G	(%)	Odds ratio*	95% CI*	
Age at diagnosis (SD)	36.0 (± 14.1)		36.3 (± 14.4)		33.9 (± 12.9)		35.5 (± 10.4)				
Male gender	401	(52.8)	346/ 655	(52.8)	52/ 100	(52.0)	3/ 4	(75.0)			
Localization (Montreal) (n=721)											
E1 (Proctitis)	124	(17.2)	110/ 623	(17.7)	14/ 95	(14.7)	0/ 3	(0)	0.78	0.43	1.42
E2 (Left sided)	245	(44.0)	212	(34.0)	33	(34.7)	0	(0)	0.98	0.63	1.54
E3 (Extended/ pancolitis)	352	(48.8)	301	(48.3)	48	(50.5)	3	(100)	1.16	0.76	1.79
Extraintestinal disease (n=228)	42	(18.4)	33/183	(18.0)	8/ 43	(18.6)	1/ 2	(50)	1.14	0.50	2.59
Surgery (n=759)	145	(19.1)	120/ 655	(18.3)	24/ 100	(24.0)	1/ 4	(25.0)	1.41	0.86	2.31
Malignancy (n=384)	2	(0.5)	1/ 329		1/ 53	(1.9)	0/ 2	(0)	6.07	0.37	98.57
Family diagnosis of IBD (n=547)	81	(14.8)	72/ 472	(0.3)	9/ 72	(12.5)	0/ 3	(0)	0.76	0.36	1.59

*Carriers of the mutant allele (T/G and G/G) were compared to wild-types (T/T).
Values are presented as absolute numbers (percentages).

As shown by immunohistochemical staining of intestinal tissue, dectin-1 is mainly present on macrophages, but also weakly on epithelial and endothelial layers of the intestine. Similar findings in mice have been demonstrated by Wong and co-workers, who demonstrated that dectin-1 is mainly expressed on populations of myeloid cells (monocyte/macrophage and neutrophil lineages).[22] In addition, they demonstrated that dectin-1 is also expressed in the Peyer's patches and along the lamina propria of the mouse intestine.[22,23] Interestingly, dectin-1 expression appeared to be elevated in inflamed intestinal tissue compared to normal tissue, due to the increased infiltration of immune cells and increased dectin-1 expression on the cell membrane of immune cells.

However, intestinal expression of dectin-1 did not appear to be disease specific but rather dependent on influx of macrophages. In fact, expression of dectin-1 was also present in intestinal samples from patients with diverticulitis. Increased infiltration of macrophages in severe diverticulitis showed an increased expression of dectin-1, compared to mild diverticulitis which is accompanied by less infiltration of macrophages.

Cohorts of CD (n = 778) and UC (n = 759) patients were screened for the *DECTIN-1* c.714T>G polymorphism and compared to a group of healthy subjects (n = 772). Subsequently, these genetic data were correlated with clinical parameters reflecting disease severity. This analysis revealed no statistical significant association between the prevalence of the *DECTIN-1 c.714G* allele and IBD, neither in disease occurrence nor in disease severity. However, one can observe that homozygous individuals bearing the *DECTIN-1* polymorphism were twice as frequent in healthy controls compared to IBD patients. This may suggest that complete absence of dectin-1 function could protect against IBD. It is important to realize that the only two dectin-1 isoforms capable of binding β-glucans (isoforms A and B) are structurally equally affected by the *DECTIN-1* c.714T>G polymorphism.[24] The occurrence of splicing isoforms with residual function could therefore be excluded. The potential mechanism of protection are likely to include the lower production of pro-inflammatory cytokines, including IL-17, in the individuals with defective dectin-1. In these series, statistical power to preclude any functional difference is insufficient due to the low prevalence of homozygous individuals. Additional studies in homozygous and heterozygous subpopulations are needed to confirm the reported observations.

All together, the reports of the association of mutations within *NOD2* and *CARD9* in patients with CD, the presence of ASCA, and the shared signaling pathway of dectin-1 and NOD2, points toward a possible link between NOD2 and dectin-1.[25] Since mutations in *NOD2* in CD patients are associated with ileal involvement and increased need for surgery and stricturing disease,[26] a potential interaction between *NOD2* mutations and the *DECTIN-1* c.714T>G polymorphism with regard to phenotypical characteristics was investigated. However, no statistical interaction could be demonstrated (data not shown).

Our data demonstrate that dectin-1 expression is elevated on macrophages, neutrophils, and other immune cells involved in the inflammatory reaction in

IBD. The *DECTIN-1* c.714T>G polymorphism is not a major susceptibility factor for protection against IBD, although a trend towards a lower frequency of the polymorphism in CD and UC cohorts was observed, in particular in the number of individuals homozygous for the *DECTIN-1* polymorphism. These genetic findings warrant further investigation of this pathogenetic pathway.

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References

1. Sartor RB. Microbial influences in inflammatory bowel diseases. Gastroenterology 2008;34:577-594.
2. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448:427-434.
3. Tysk C, Lindberg E, Jarnerot G, et al. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. Gut 1988;29:990-996.
4. Cho JH, Weaver CT. The genetics of inflammatory bowel disease. Gastroenterology 2007;133:1327-1339.
5. Girardin SE, Boneca IG, Viala J, et al. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 2003;278:8869-8872.
6. Inohara N, Ogura Y, Fontalba A, et al. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. J Biol Chem 2003;278:5509-5512.
7. Gross O, Gewies A, Finger K, et al. Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. Nature 2006;442:651-656.
8. Hsu YM, Zhang Y, You Y, et al. The adaptor protein CARD9 is required for innate immune responses to intracellular pathogens. Nat Immunol 2007;8:198-205.
9. LeibundGut-Landmann S, Gross O, Robinson MJ, et al. Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. Nat Immunol 2007;8:630-638.
10. Zhernakova A, Festen EM, Franke L, et al. Genetic analysis of innate immunity in Crohn's disease and ulcerative colitis identifies two susceptibility loci harboring CARD9 and IL18RAP. Am J Hum Genet 2008;82:1202-1210.
11. Ferwerda B, Ferwerda G, Plantinga TS, et al. A family with human dectin-1 deficiency and mucocutanous fungal infections. N Engl J Med 2009;361:1760-1767.
12. Plantinga TS, van der Velden WJ, Ferwerda B, et al. Early stop polymorphism in human DECTIN-1 is associated with increased candida colonization in hematopoietic stem cell transplant recipients. Clin Infect Dis 2009;49:724-732.
13. Seiderer J, Elben I, Diegelmann J, et al. Role of the novel th17 cytokine IL-17F in inflammatory bowel disease (IBD): Upregulated colonic IL-17F expression in active Crohn's disease and analysis of the IL17F p.Hisl6lArg polymorphism in IBD. Inflamm Bowel Dis 2008;14:437-445.
14. van Beelen AJ, Zelinkova Z, Taanman-Kueter EW, et al. Stimulation of the intracellular bacterial sensor NOD2 programs dendritic cells to promote interleukin-17 production in human memory T cells. Immunity 2007;27:660-669.
15. Standaert-Vitse A, Jouault T, Vandewalle P, et al. Candida albicans is an immunogen for anti-Saccharomyces cerevisiae antibody markers of Crohn's disease. Gastroenterology 2006;130:1764-1775.
16. Quinton JF, Sendid B, Reumaux D, et al. Anti-Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. Gut 1998;42:788-791.
17. Podolsky DK. Inflammatory bowel disease. New Engl J Med 2002;347:417-429
18. Gasche C, Scholmerich J, Brynskov J, et al. A simple classification of Crohn's disease: Report of the Working Party for the world congresses of gastroenterology, Vienna 1998. Inflamm Bowel Dis 2000;6:8-15..

19. Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol 2005;19 Suppl A:5-36.
20. Oostenbrug LE, Nolte IM, Oosterom E, et al. CARD15 in inflammatory bowel disease and Crohn's disease phenotypes: An association study and pooled analysis. Digestive and Liver Disease 2006;38:834-845.
21. Ferwerda G, Meyer-Wentrup F, Kullberg BJ, et al. Dectin-1 synergizes with TLR2 and TLR4 for cytokine production in human primary monocytes and macrophages. Cell Microbiol 2008;10:2058-2066.
22. Taylor PR, Brown GD, Reid DM, et al. The beta-glucan receptor, dectin-1, is predominantly expressed on the, surface of cells of the monocyte/macrophage and neutrophil lineages. J Immunol 2002;169:3876-3882.
23. Reid DM, Montoya M, Taylor PR, et al. Expression of the beta-glucan receptor, dectin-1, on murine leukocytes in situ correlates with its function in pathogen recognition and reveals potential roles in leukocyte interactions. J Leukoc Biol 2004;76:86-94.
24. Willment JA, Gordon S, Brown GD. Characterization of the human beta -glucan receptor and its alternatively spliced isoforms. J Biol Chem 2001;276:43818-43823.
25. Underhill D, Braun J. Current understanding of fungal microflora in inflammatory bowel disease pathogenesis. Inflamm Bowel Dis 2008;14:1147-1153.
26. Russell RK, Drummond HE, Nimmo EE, et al. Genotype-phenotype analysis in childhood-onset Crohn's disease: NOD2/CARD15 variants consistently predict phenotypic characteristics of severe disease. Inflamm Bowel Dis 2005;11:955-964.

Chapter 7

The functional -765G→C polymorphism of the *COX-2* gene may reduce the risk of developing Crohn's disease

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Cyclooxygenase-2 (COX-2) is a key enzyme involved in the conversion of arachidonic acid into prostaglandins. COX-2 is mainly induced at sites of inflammation in response to proinflammatory cytokines such as interleukin-1α/β, interferon-γ and tumor necrosis factor-α produced by inflammatory cells.
The aim of this study was to investigate the possible modulating effect of the functional *COX-2* polymorphisms -1195A→G and -765G→C on the risk for development of inflammatory bowel disease (IBD) in a Dutch population.
Genomic DNA of 525 patients with Crohn's disease (CD), 211 patients with ulcerative colitis (UC) and 973 healthy controls was genotyped for the -1195 A→G (rs689466) and -765G→C (rs20417) polymorphisms. Distribution of genotypes in patients and controls were compared and genotype-phenotype interactions were investigated.
The genotype distribution of the -1195A→G polymorphism was not different between the patients with CD or UC and the control group. The -765GG genotype was more prevalent in CD patients compared to controls with an OR of 1.33 (95% CI 1.04-1.69, $p < 0.05$). The -765GC and -765CC genotype carriers showed a tendency to be less frequent in patients with CD compared to controls, with ORs of 0.78 (95% CI: 0.61-1.00) and 0.49 (95% CI 0.22-1.08), respectively. Combining homozygous and heterozygous patients with the -765C allele showed a reduced risk for developing CD, with an OR of 0.75 (95% CI: 0.59-0.96). In the context of this, the $G_{-1195}G_{-765}/A_{-1195}C_{-765}$ diplotype was significantly less common in patients with CD compared to controls, with an OR of 0.62 (95% CI: 0.39-0.98). For UC however, such an effect was not observed. No correlation was found between COX-2 diplotypes and clinical characteristics of IBD.
In conclusion, the -765G→C polymorphism was associated with a reduced risk for developing Crohn's disease in a Dutch population.

Introduction

Inflammatory bowel disease (IBD) is an idiopathic, chronic, relapsing auto inflammatory disorder of the gastro-intestinal tract. The two major types of IBD are Crohn's disease (CD) and ulcerative colitis (UC). Genetic, immunological and environmental factors are thought to play a role in the pathogenesis of IBD.[1] A dysregulated immune response against the intestinal microbiota in genetic susceptible individuals has been heavily implicated in the pathogenesis of inflammatory bowel disease.[2] Therefore, genes involved in inflammatory responses are under investigation to look for variants predisposing to IBD.

Cyclooxygenase (COX) is a modifier gene and key enzyme in the conversion of free arachidonic acid into prostaglandins and is involved in the regulation of inflammatory processes through its products, mainly prostaglandin E2 (PGE2).[3] The COX family consists of two main isozymes: COX-1 and COX-2. COX-1 is constitutively expressed in most cell types, including the mucosal compartment of the gastrointestinal tract, and is important for maintaining mucosal integrity, mucosal defence and regulation of the mucosal blood flow.[4, 5] Being very low expressed in the normal gut mucosa, COX-2 expression can be induced by mitogenic and proinflammatory stimuli.[5, 6]

The relevance of COX-2 in the pathogenesis of IBD has been demonstrated; increased expression of COX-2 has been observed in colonic epithelial cells, the myenteric plexus and in the medial layer of arteries from patients with active IBD.[7–9] In addition, a relationship between endoscopic activity of IBD and mucosal COX-2 mRNA levels was noticed.[10]

Although COX-2 is involved in the regulation of inflammatory processes, it also seems to play a physiological role in the defence of the gastric mucosa, as well as in the maintenance of gastric mucosal integrity when other defence mechanisms are impaired or COX-1 activity is latent.[3, 5] Moreover, COX-2 seems to be a major contributor to the processes that lead to resolution of inflammation.[11] In line with this, the use of non-steroidal anti-inflammatory drugs (NSAIDs) in patients with IBD may be associated with exacerbation of the underlying IBD and gastrointestinal-related complications.[12–14] Overall, these findings suggest that COX-2 has a dual role by both initiation as well as resolution of inflammation.

Functional polymorphisms in the *COX-2* promoter, being -765G→C (rs20417) and -1195A→G (rs689466), may alter the enzyme function of COX-2 by differential regulation of COX-2 expression.[15] Recently, a study by Østergaard *et al.* reported an association of the -765G→C polymorphism with IBD in a Danish population.[16] Another study from a previous relatively small sample size study performed in the Netherlands however, showed no association between these two polymorphisms and IBD.[17] We therefore investigated the *COX-2* -1195A→G and -765G→C polymorphisms in relation to the development and clinical severity of IBD in a phenotypically well characterized and relatively large IBD cohort of Dutch origin and hypothesized that carriers of the -1195A→G and/or -765G→C polymorphisms might be at risk for developing IBD.

Materials and Methods

Patients and controls

This case-control study included 736 patients with inflammatory bowel disease (39% men, mean age 45.0 ± 13.9 years), 525 patients with Crohn's disease (35% men, mean age 44.5 ± 13.9) and 211 patients with ulcerative colitis (48% men, mean age 46.1 ± 14.0) and 973 disease-free controls (43% men, mean age 47.2 ± 16.6 years). All patients were of Dutch origin and were recruited from the outpatient clinic of the Radboud University Nijmegen Medical Center, the Netherlands. Controls were recruited from the Nijmegen area by advertisement in local papers. The clinical characteristics of the patients are summarized in tables 7.1 and 7.2. Diagnosis of inflammatory bowel disease was based on accepted clinical, endoscopic, radiological and histological findings.[18] Clinical data of the patients were retrieved by retrospective collection from patients' clinical charts. Phenotypes of the patients were described according to age of onset, necessity of surgery, family history of IBD, the occurrence of extra-intestinal manifestations and maximum extent of disease according to the Vienna[19] and Montreal[20] classifications for Crohn's disease and ulcerative colitis respectively. Information on development of dysplasia and colorectal cancer (CRC) in our patient cohort was retrieved using PALGA, the nationwide network and registry of histopathology and cytopathology in the Netherlands.[21]

The ethical committee of region Nijmegen and Arnhem reviewed and approved the protocol under number CWOM-nr 9804-0100. Verbal informed consent was obtained from each patient before study participation in agreement with the approval and all samples were anonymized. Since research data were collected anonymously, at least verbal informed consent was needed according to national regulations. Therefore, no written informed consent procedure was introduced at time of data collection.

Genotyping

Whole blood from patients and healthy controls was obtained by venapuncture in sterile vacutainer tubes, anti-coagulated with EDTA and stored at -20°C until use. DNA from patients and controls was isolated from whole blood using the Pure Gene DNA isolation kit, according to the instructions of the manufacturer (Gentra Systems, Minneapolis, MN) and stored at 4°C. Genotypes of the *COX-2* -1195A→G polymorphism was determined by polymerase chain reaction (PCR)-based restriction fragment length polymorphism assays, as described by Zhang *et al.*[15] The *COX-2* -765G→C polymorphism was determined by a dual-color discrimination assay using the iCycler iQ Multicolour Real-Time Detection System (Bio-Rad Laboratories, Hercules, CA), as described by Peters *et al.*[22]

Statistical analysis

Baseline and clinical characteristics were analysed with standard descriptive statistics. The observed genotype frequencies were tested for deviation from the Hardy-Weinberg equilibrium.

Estimates of linkage disequilibrium (LD) between SNPs were determined by calculating pair-wise D^2 and r^2 statistics in unrelated individuals, using Haploview. Differences in -1195A→G and -765G→C genotype distributions between the patient and control groups were determined by χ^2 analysis. Odds ratios (ORs) with 95% confidence interval (95% CI) were calculated for genotypes associated with predicted normal versus predicted altered enzyme activities (variant genotypes) between IBD patients and controls. These analyses were also applied for testing of either UC or CD with the control group. Based on the two polymorphisms investigated, a diplotype analysis was performed. Diplotypes were compared with regard to phenotypical characteristics and comparisons were given as ORs with 95% CI. Additionally, we investigated in patients with IBD whether the -1195A→G and -765G→C polymorphisms were associated with development of mucosal dysplasia or colon cancer. Data analysis was performed using SPSS software (Version 16.0, SPSS, Chicago, IL, USA). A p-value of < 0.05 was used as a criterion for statistical significance.

Table 7.1 Clinical characteristics of patients with Crohn's disease (n=525)

Characteristics (%)	
Age at diagnosis, years (SD)	27.1 ± 10.7
Family history of IBD*	75/272 (27.6)
Disease localization	
Ileal	187 (35.6)
Colonic	127 (24.2)
Ileocolonic	211 (40.2)
Isolated upper disease+	36 (6.9)
Disease behaviour CD	
Non stricturing, non penetrating	176 (33.5)
Stricturing	89 (17.0)
Penetrating	260 (49.5)
Extra-intestinal disease*	161/ 491 (32.8)
Peri-anal disease*	180/ 509 (35.4)
Surgery	320 (61.0)

+Patients could be classified as having disease localisation in the upper gastrointestinal tract next to ileal, colonic or ileocolonic localisation. *Note that data of patients are missing

Table 7.2 Clinical characteristics of patients with Ulcerative Colitis (n=211)

Characteristics (%)	
Age at diagnosis, years (SD)	32.9 ± 12.7
Disease localization*	
Proctitis	13/ 203 (6.4)
Left sided	70/ 203 (34.5)
Extended/pancolitis	120/ 203 (59.1)
Surgery	59 (28.0)

*Note that data of 8 patients are missing

Results

In this study 736 patients with inflammatory bowel disease, 525 patients with Crohn's disease and 211 patients with ulcerative colitis as well as 973 healthy controls were included. No statistical significant differences were observed between patients with IBD and controls regarding age and gender. However when the CD or UC patient groups were compared to controls separately, significant more females were present in the group with Crohn's disease ($p < 0.01$).

Distribution of the -1195 and -765 *COX-2* genotypes in both patient and control groups fitted the Hardy Weinberg equilibrium; for the -1195 *COX-2* genotypes, p-values of $p = 0.14$, $p = 0.17$ and $p = 0.99$, for the patients with Crohn's disease, ulcerative colitis and controls were found; whereas corresponding p-values for the -765 *COX-2* genotypes were $p = 0.64$, $p = 0.26$ and $p = 0.87$, respectively. As been reported before by others,[15, 17, 23] both SNPs were found to be in strong linkage disequilibrium ($D' = 1$, $r^2 = 0.05$).

Genotype distribution and association with inflammatory bowel disease

The distribution of the -1195 and -765 *COX-2* genotypes as found in patients with IBD and controls is given in table 7.3. The -1195 genotype distribution was not different between the patients with Crohn's disease, ulcerative colitis, or all IBD patients taken together in comparison with the control group. However, the -765 genotype distribution showed a tendency towards a significant difference between patients with Crohn's disease and controls, with the -765GC and -765CC genotypes being less prevalent in patients, with ORs of 0.78 (95% CI 0.61-1.00, $p < 0.05$) and 0.49 (95% CI 0.22-1.08) respectively and the -765GG genotype being more prevalent in patients (OR 1.33, 95% CI 1.04-1.69, $p < 0.05$). No differences were found between patients with ulcerative colitis and controls. Combining homozygous (-765CC) and heterozygous (-765GC) patients bearing the -765C allele, showed a reduced risk for developing Crohn's disease in this group (OR = 0.75, 95% CI 0.59-0.96, $p < 0.05$).

The effects of the two *COX-2* polymorphisms were then studied in the context of diplotypes. Six diplotypes were identified, with the $A_{-1195}G_{-765}/A_{-1195}G_{-765}$ diplotype being the most prevalent in both patients and controls (table 7.4). The $G_{-1195}G_{-765}/A_{-1195}C_{-765}$ diplotype was significantly less frequent in patients with Crohn's disease compared to controls with an OR of 0.62 (95% CI 0.39-0.98, $p < 0.05$).

Correlation of the COX-2 diplotypes with clinical characteristics of IBD patients

Additionally, clinical characteristics of patients with Crohn's disease and ulcerative colitis were studied in the context of diplotypes in which the most common $A_{-1195}G_{-765}/A_{-1195}G_{-765}$ diplotype served as reference. No significant association between the *COX-2* diplotypes and clinical characteristics of either Crohn's disease or ulcerative colitis was found (tables 7.5 and 7.6). When data were corrected for age and gender, no significant changes in data were observed.

Table 7.3. Distribution of the *COX-2* -1195 and -765 genotypes and corresponding ORs in patients with IBD, CD or UC versus controls

Genotype *COX-2*	All patients with IBD (n = 736)			Patients with Crohn's disease (n = 525)			Patients with Ulcerative Colitis (n = 211)[+]			Controls
	Number (%)	OR (95% CI)	p-value	Number (%)	OR (95% CI)	p-value	Number (%)	OR (95% CI)	p-value	n = 973 (%)
-1195AA	476 (64.7)	Reference	-	339 (64.6)	Reference	-	137 (64.9)	Reference	-	618 (63.5)
-1195GA	221 (30.0)	0.91 (0.74-1.12)	0.38	159 (30.3)	0.92 (0.73-1.16)	0.48	62 (29.4)	0.89 (0.64-1.23)	0.48	315 (32.4)
-1195GG	39 (5.3)	1.27 (0.80-2.00)	0.31	27 (5.1)	1.23 (0.74-2.04)	0.42	12 (5.7)	1.35 (0.69-2.65)	0.38	40 (4.1)
-765GG	535 (73.2)	Reference	-	394 (75.0)	Reference	-	141 (68.4)	Reference	-	675 (69.4)
-765GC	179 (24.5)	0.84 (0.67-1.04)	0.11	123 (23.4)	0.78 (0.61-1.00)	0.05	56 (27.2)	0.99 (0.71-1.40)	0.97	270 (27.7)
-765CC	17 (2.3)	0.77 (0.42-1.41)	0.39	8 (1.5)	0.49 (0.22-1.08)	0.07	9 (4.4)	1.53 (0.71-3.33)	0.27	28 (2.9)

[+]In the ulcerative colitis group, there are some missing data (n = 5) due to unsuccessful PCR for the -765 G→C polymorphism. OR = Odds ratio; CI = confidence interval.

Table 7.4 COX-2 diplotype distribution and corresponding ORs in patients with IBD, CD or UC versus controls

Diplotype *COX-2*	All patients with IBD (n = 731)			Patients with Crohn's disease (n = 525)			Patients with Ulcerative Colitis (n = 206)			Controls
	Number (%)	OR (95% CI)	p-value	Number (%)	OR (95% CI)	p-value	Number (%)	OR (95% CI)	p-value	n = 973 (%)
$A_{-1195}G_{-765}/A_{-1195}G_{-765}$	322 (43.8)	Reference	-	237 (45.1)	Reference	-	85 (40.3)	Reference	-	395 (40.6)
$G_{-1195}G_{-765}/A_{-1195}G_{-765}$	174 (23.6)	0.90 (0.70-1.15)	0.38	130 (24.8)	0.91 (0.70-1.19)	0.49	44 (20.9)	0.86 (0.58-1.28)	0.45	238 (24.5)
$A_{-1195}G_{-765}/A_{-1195}C_{-765}$	133 (18.1)	0.84 (0.65-1.10)	0.20	94 (17.9)	0.81 (0.60-1.08)	0.15	39 (18.5)	0.93 (0.62-1.42)	0.75	194 (19.9)
$G_{-1195}G_{-765}/A_{-1195}C_{-765}$	46 (6.5)	0.72 (0.49-1.07)	0.11	29 (5.5)	0.62 (0.39-0.98)	0.04	17 (8.1)	1.01 (0.57-1.80)	0.97	78 (8.0)
$G_{-1195}G_{-765}/G_{-1195}G_{-765}$	39 (5.3)	1.20 (0.75-1.90)	0.45	27 (5.1)	1.13 (0.67-1.88)	0.65	12 (5.7)	1.39 (0.70-2.77)	0.34	40 (4.1)
$A_{-1195}C_{-765}/A_{-1195}C_{-765}$	17 (2.3)	0.75 (0.40-1.39)	0.35	8 (1.5)	0.48 (0.21-1.06)	0.06	9 (4.3)	1.49 (0.68-3.28)	0.32	28 (2.9)

OR = Odds ratio; CI = confidence interval.

Table 7.5 Diplotype-phenotype correlations in patients with Crohn's disease

	AG/AG*	GG/AG	OR (95% CI)	p-value	AG/AC	OR (95% CI)	p-value	GG/AC	OR (95% CI)	p-value	GG/GG	OR (95% CI)	p-value	AC/AC	OR (95% CI)	p-value
Disease localization (n = 525)																
Ileal	83	51	Reference	-	34	Reference	-	8	Reference	-	8	Reference	-	3	Reference	-
Colonic	63	26	0.67 (0.38 - 1.19)	0.17	23	0.89 (0.48 - 1.66)	0.72	8	1.32 (0.47 - 3.70)	0.60	6	0.99 (0.33 - 2.99)	0.98	1	0.44 (0.05 - 4.32)	0.47
Ileocolonic	91	53	0.95 (0.58 - 1.54)	0.83	37	0.99 (0.57 - 1.73)	0.98	13	1.48 (0.59 - 3.76)	0.41	13	1.48 (0.59 - 3.76)	0.41	4	1.22 (0.26 - 5.60)	0.80
Isolated upper disease+	18	7	0.63 (0.25 - 1.62)	0.34	8	1.09 (0.43 - 2.73)	0.86	2	1.15 (0.23 - 5.89)	0.86	1	0.58 (0.07 - 4.90)	0.61	0	-	0.42
Disease behavior (n = 525)																
Non stricturing/ penetrating	75	44	Reference	-	37	Reference	-	10	Reference	-	8	Reference	-	2	Reference	-
Stricturing	43	18	0.71 (0.37 - 1.39)	0.32	14	0.66 (0.32 - 1.36)	0.26	8	1.40 (0.51 - 3.80)	0.51	4	0.87 (0.25 - 3.07)	0.83	2	1.74 (0.24 - 12.83)	0.58
Penetrating	119	68	0.97 (0.61 - 1.57)	0.91	43	0.73 (0.43 - 1.24)	0.25	11	0.69 (0.28 - 1.71)	0.43	15	1.18 (0.48 - 2.92)	0.72	4	1.26 (0.23 - 7.05)	0.79
Surgery	146	87	1.26 (0.81 - 1.98)	0.31	50	0.71 (0.44 - 1.15)	0.16	16	0.77 (0.35 - 1.67)	0.50	17	1.06 (0.47 - 2.42)	0.89	4	0.62 (0.15 - 2.55)	0.51
Extra-intestinal (n=491)	77	32	0.69 (0.43 - 1.13)	0.14	37	1.39 (0.84 - 2.30)	0.21	8	0.80 (0.34 - 1.92)	0.62	6	0.64 (0.24 - 1.67)	0.36	1	0.27 (0.03 - 2.26)	0.20
Family history of IBD (n=272)	36	18	0.82 (0.43 - 1.59)	0.57	14	1.08 (0.52 - 2.26)	0.84	2	0.62 (0.13 - 3.05)	0.55	3	0.67 (0.18 - 2.56)	0.56	2	1.65 (0.26 - 10.28)	0.59
Dysplasia	7	4	1.04 (0.30 - 3.63)	0.95	1	0.35 (0.04 - 2.91)	0.31	1	1.17 (0.14 - 9.89)	0.88	2	2.63 (0.52 - 13.35)	0.23	0	-	0.62
Colon cancer	4	3	0.73 (0.16 - 3.30)	0.68	0	-	0.21	0	-	0.48	0	-	0.50	0	-	0.71

Diplotype-phenotype correlations in patients with Crohn's disease in which the AG/AG diplotype served as reference.
+Patients could be classified as having disease localisation in the upper gastrointestinal tract next to ileal, colonic or ileocolonic localisation.
*For full notation of the diplotypes see Table 7.4.
OR = Odds ratio; CI = confidence interval.

Table 7.6 Diplotype-phenotype correlations in patients with ulcerative colitis.

	AG/AG*	GG/AG	OR (95% CI)	p-value	AG/AC	OR (95% CI)	p-value	GG/AC	OR (95% CI)	p-value	GG/GG	OR (95% CI)	p-value	AC/AC	OR (95% CI)	p-value
Disease localization (n=198)																
Proctitis	6	2	Reference	-	2	Reference	-	2	Reference	-	1	Reference	-	0	Reference	-
Left sided	27	13	1.44 (0.26 - 8.16)	0.68	15	1.67 (0.30 - 9.31)	0.56	7	0.78 (0.13 - 4.72)	0.78	3	0.67 (0.06 - 7.57)	0.74	4	-	0.35
Pancolitis	46	28	1.83 (0.35 - 9.68)	0.47	21	1.37 (0.26 - 7.36)	0.71	8	0.52 (0.09 -3.06)	0.47	8	1.04 (0.11 - 9.86)	0.97	5	-	0.42
Surgery	24	11	0.85 (0.37 - 1.94)	0.70	11	1.00 (0.43 - 2.32)	1.0	8	2.26 (0.78 - 6.54)	0.13	2	0.51 (0.10 - 2.49)	0.40	2	0.73 (0.14 - 3.75)	0.70
Dysplasia	5	3	1.17 (0.27 - 5.14)	0.84	2	0.87 (0.16 - 4.67)	0.87	0	-	0.31	1	1.46 (0.16 - 13.63)	0.74	2	4.57 (0.75 - 28.01)	0.08

Diplotype-phenotype correlations in patients with ulcerative colitis in which the AG/AG diplotype served as reference.
*For full notation of the diplotypes see Table 7.4.
OR = Odds ratio; CI = confidence interval.

COX-2 polymorphisms and the risk for developing dysplasia and colon cancer in patients with inflammatory bowel disease

The PALGA search regarding dysplasia and colon cancer in our IBD cohort demonstrated that 29 patients (15 patients with CD and 14 patients with UC) developed mucosal dysplasia, which is regarded as a pre-malignant phase of CRC. Furthermore, in the CD cohort 7 patients with CRC were identified; 4 having the $A_{-1195}G_{-765}/A_{-1195}G_{-765}$ diplotype and 3 having the $G_{-1195}G_{-765}/A_{-1195}G_{-765}$ diplotype. In the UC cohort, no patients were identified who developed CRC. When tested, no association was found between the *COX-2* diplotypes and the development of colonic dysplasia or cancer (tables 7.5 and 7.6).

Discussion

This study was performed to determine the possible modulating effect of the *COX-2* -1195 A→G and -765G→C polymorphisms on the risk of developing inflammatory bowel disease. Carriers of the -765C allele showed a reduced risk for developing CD. This result suggests that the -765G→C change induces an altered enzyme expression and enzyme activity with potential anti-inflammatory consequences.

Studies regarding the functional consequences of the -765G→C polymorphism in the *COX-2* promoter are conflicting. Therefore, the (physiological) consequences of our findings are difficult to interpret. First of all, the -765C-containing *COX-2* promoter was reported to drive lower reporter gene expression in vitro compared to the -765G-containing counterpart.[15, 24] Furthermore, serum prostaglandin E_2 (PGE_2) concentrations of renal transplant recipients patients with the GG genotype were significantly higher than PGE_2 concentrations from patients with the C allele.[25] Subsequent work from Zhang and coworkers showed that the -765G→C polymorphism creates a binding site for nucleophosmin (NPM) and phosphorylated nucleophosmin (p-NPM), which acts as an inhibitor of COX-2 transcription.[26] The -1195 A→G polymorphism creates a c-MYB binding site, which can activate COX-2 expression, and displays a higher promoter activity.[15]

In normal colorectal mucosa COX-2 expression is enhanced in patients with IBD when compared to subjects with normal colonoscopy.[27] Taken together in light of our results, this would imply that low levels of COX-2 are associated with an reduced risk for developing CD. In vitro however, when cells were treated with smoking condensate, the -765C-containing promoter exerted a significantly higher reporter gene expression compared to the -765G-containing counterpart.[26] Besides this, Szczeklik and co-workers reported an increased production of prostaglandin E_2 and D_2 (PGE_2 and PGD_2) by monocytes obtained from female patients with asthma who were homozygous for the -765C variant of the *COX-2* gene.[28, 29] In the context of IBD, PGE_2 appears to play a dual role. In IBD, PGE_2 production is increased[30] and in an experimental model of IBD high levels of PGE_2 exacerbate inflammation.[31] On the other hand, PGE_2 signaling is required for suppressing colitis symptoms and protecting mucosal damage by maintaining the integrity of the epithelial intestinal wall, presumably through the enhancement of epithelial survival and regeneration.[32]

Furthermore, PGE_2 has been recently identified to promote naive T cell differentiation to IL-17 – producing T helper (Th17) cells, a subset of T helper cells which have been implicated as potent effector cells in IBD.[33]

Several limitations of our study should be noticed. First of all we were not able to retrieve the smoking status of our patients and controls, as Zhao *et al.*[26] demonstrated an effect of smoking on the expression of the -765G→C polymorphism. Secondly, the effect of the *COX-2* -1195A→G and -765G→C polymorphisms on colonic mucosal COX-2 expression and/or PGE_2 production in patients with IBD is unknown. However regardless of these data, the functional consequences of PGE_2 in IBD still remains conflicting as pointed out above.

The results of our study are in conflict with a Danish case control study by Østergaard *et al.* who identified that carriers of the homozygous -765CC variant had a relatively high risk for developing CD as well as UC, with an OR of 2.78 (95% CI = 1.33-5.88, p = 0.006) and 2.63 (95% CI = 1.35-5.26, p = 0.005) respectively.[16] The -765CC variant however is very rare in our population of IBD patients (n = 17, 2.3%) and controls (n = 28, 2.9%) as is the case in another Dutch study by Cox *et al.* in which (2.4%) of the patients and (2.4%) of the controls had this variant.[17] In the study of Cox *et al.*, no significant association between the -1195A→G and -765G→C polymorphisms and IBD was found, although the number of patients with IBD involved (n = 291) was rather small. However, a recent subsequent study from the Danish group of Østergaard and co-workers extended the original data with data from Scottish IBD patients and showed no association any more with the -765G→C polymorphism and development of IBD.[34] The differences between our results and the Danish and Scottish findings could be attributed to the fact that the genetical contribution to the etiology of IBD in the northern part of Europe differs from central Europe. Mutations in the three common CD-associated variants of *CARD15*, R702W, G908R and 1007fsinsC, are relatively rare in Northern countries including Denmark and Scotland, while the mutation frequencies are relatively high in Central Europe.[35]

As stated before, patients with IBD show increased expression of COX-2 in the gastrointestinal tract.[7,8,10,27] This increased expression of COX-2 has also been observed in gastrointestinal adenocarcinomas and in UC-associated neoplasia.[36,37] Additionally, the *COX-2* -1195A→G and -765G→C polymorphisms were demonstrated to influence the expression of COX-2 and confer a risk for developing (adeno)carcinomas in the gastrointestinal tract.[15, 38, 39] Chronic intestinal inflammation-associated colorectal carcinogenesis is thought to occur via a stepwise progression beginning with epithelial hyperplasia, leading to various grades of dysplasia, adenoma, and then to adenocarcinoma.[40] We investigated whether or not an association could be found between the COX-2 polymorphisms and dysplasia or CRC in patients with IBD. Due to the restricted number of patients who developed dysplasia or CRC, no differences could be observed.

Conclusion

Subjects with the -765C allele showed a reduced risk for developing CD. No correlation could be found between the *COX-2* diplotypes and clinical characteristics of IBD patients and the development of colonic dysplasia or cancer. Further studies are required to confirm the association we found and efforts should be made to unravel the role of COX-2 and its derived prostaglandins in the pathogenesis of IBD.

References

1. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448:427-434.
2. Sartor RB. Microbial influences in inflammatory bowel diseases. Gastroenterology 2008;134:577-94.
3. Warner TD, Mitchell JA. Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. Faseb Journal 2004;18:790-804.
4. Martin GR, Wallace JL. Gastrointestinal inflammation: A central component of mucosal defense and repair. Exp Biol Med 2006;231:130-137.
5. Fornai M, Antonioli L, Colucci R, et al. Emerging role of cyclooxygenase isoforms in the control of gastrointestinal neuromuscular functions. Pharmacol Therapeut 2010;125:62-78.
6. Trifan OC, Hla T. Cyclooxygenase-2 modulates cellular growth and promotes tumorigenesis. J Cell Mol Med 2003;7:207-222.
7. Singer II, Kawka DW, Schloemann S, et al. Cyclooxygenase 2 is induced in colonic epithelial cells in inflammatory bowel disease. Gastroenterology 1998;115:297-306.
8. Roberts PJ, Morgan K, Miller R, et al. Neuronal COX-2 expression in human myenteric plexus in active inflammatory bowel disease. Gut 2001;48:468-472.
9. Tabernero A, Reimund JM, Chasserot S, et al. Cyclooxygenase-2 expression and role of vasoconstrictor prostanoids in small mesenteric arteries from patients with Crohn's disease. Circulation 2003;107:1407-1410.
10. Hendel J, Nielsen OH. Expression of cyclooxygenase-2 mRNA in active inflammatory bowel disease. Am J Gastroenterol 1997;92:1170-1173.
11. Wallace JL COX-2: A pivotal enzyme in mucosal protection and resolution of inflammation. The scientific world journal 2006;6:577-588.
12. Matuk R, Crawford J, Abreu MT, et al. The spectrum of gastrointestinal toxicity and effect on disease activity of selective cyclooxygenase-2 inhibitors in patients with inflammatory bowel disease. Inflamm Bowel Dis 2004;10:352-356.
13. Takeuchi K, Smale S, Premchand P, et al. Prevalence and mechanism of nonsteroidal anti-inflammatory drug-induced clinical relapse in patients with inflammatory bowel disease. Clin Gastroenterol Hepatol 2006;4:196-202.
14. Biancone L, Tosti C, Geremia A, et al. Rofecoxib and early relapse of inflammatory bowel disease: an open-label trial. Aliment Pharm Ther 2004;19:755-764.
15. Zhang XM, Miao XP, Tan W, et al. Identification of functional genetic variants in cyclooxygenase-2 and their association with risk of esophageal cancer. Gastroenterology 2005;129:565-576.
16. Ostergaard M, Ernst A, Labouriau R, et al. Cyclooxygenase-2, multidrug resistance 1, and breast cancer resistance protein gene polymorphisms and inflammatory bowel disease in the Danish population. Scand J Gastroenterol 2009;44:65-73.
17. Cox DG, Crusius JB, Peeters PH, et al. Haplotype of prostaglandin synthase 2/cyclooxygenase 2 is involved in the susceptibility to inflammatory bowel disease. World J Gastroenterol 2005;11:6003-6008.
18. Podolsky DK. Inflammatory bowel disease. New Engl J Med 2002;347:417-429.
19. Gasche C, Scholmerich J, Brynskov J, et al. A simple classification of Crohn's disease: Report of the Working Party for the world congresses of gastroenterology, Vienna 1998. Inflamm Bowel Dis 2000;6:8-15.

20. Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol 2005;19:5A-36A.
21. Casparie M, Tiebosch ATMG, Burger G, et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. Cell Oncol 2007;29:19-24.
22. Peters WH, Lacko M, Morsche RH, et al. Cox-2 Polymorphisms and the Risk for Head and Neck Cancer in White Patients. Head Neck 2009;31:938-943.
23. Rudock ME, Liu Y, Ziegier JT, et al. Association of polymorphisms in cyclooxygenase (COX)-2 with coronary and carotid calcium in the Diabetes Heart Study. Atherosclerosis 2009;203:459-465.
24. Papafili A, Hill MR, Brull DJ, et al. Common promoter variant in cyclooxygenase-2 represses gene expression - Evidence of role in acute-phase inflammatory response. Arterioscl Throm Vas 2002;22:1631-1636.
25. Courivaud C, Bamoulid J, Loupy A, et al. Influence of Cyclooxygenase-2 (COX-2) Gene Promoter Polymorphism-765 on Graft Loss After Renal Transplantation. Am J Transplant 2009;9:2752-2757.
26. Zhao D, Xu DK, Zhang XM, et al. Interaction of Cyclooxygenase-2 Variants and Smoking in Pancreatic Cancer: A Possible Role of Nucleophosmin. Gastroenterology 2009;136:1659-1668.
27. Mariani F, Sena P, Marzona L, et al. Cyclooxygenase-2 and Hypoxia-Inducible Factor-1 alpha protein expression is related to inflammation, and up-regulated since the early steps of colorectal carcinogenesis. Cancer Lett 2009;279:221-229.
28. Szczeklik W, Sanak M, Szczeklik A. Functional effects and gender association of COX-2 gene polymorphism G(-765)C in bronchial asthma. J Allergy Clin Immun 2004;114:248-253.
29. Sanak M, Szczeklik W, Szczeklik A. Association of COX-2 gene haplotypes with prostaglandins production in bronchial asthma. J Allergy Clin Immun 2005;116:221-223.
30. Carty E, De Brabander M, Feakins RM, et al. Measurement of in vivo rectal mucosal cytokine and eicosanoid production in ulcerative colitis using filter paper. Gut 2000;46:487-492.
31. Sheibanie AF, Yen JH, Khayrullina T, et al. The proinflammatory effect of prostaglandin E-2 in experimental inflammatory bowel disease is mediated through the IL-23 -> IL-17 axis. Journal Immunol 2007;178:8138-8147.
32. Jiang GL, Nieves A, Im WB, et al. The prevention of colitis by E prostanoid receptor 4 agonist through enhancement of epithelium survival and regeneration. J Pharmacol Exp Ther 2007;320:22-28.
33. Boniface K, Bak-Jensen KS, Li Y, et al. Prostaglandin E2 regulates Th17 cell differentiation and function through cyclic AMP and EP2/EP4 receptor signaling. J Exp Med 2009;206:535-548.
34. Andersen V, Nimmo E, Krarup HB, et al. Cyclooxygenase-2 (COX-2) polymorphisms and risk of inflammatory bowel disease in a Scottish and Danish case-control study. Inflamm Bowel Dis 2011;17:937-946.
35. Hugot JP, Zaccaria I, Cavanaugh J, et al. Prevalence of CARD15/NOD2 mutations in Caucasian healthy people. Am J Gastroenterol 2007;102:1259-1267.
36. Hasegawa K, Ichikawa W, Fujita T, et al. Expression of cyclooxygenase-2 (COX-2) mRNA in human colorectal adenomas. Eur J Cancer 2001;37:1469-1474.
37. Agoff SN, Brentnall TA, Crispin DA, et al. The role of cyclooxygenase 2 in ulcerative colitis-associated neoplasia. Am J Pathol 2000;157:737-745.

38. Hoff JH, te Morsche RH, Roelofs HM, et al. COX-2 polymorphisms -765G-->C and -1195A-->G and colorectal cancer risk. World J Gastroenterol 2009;15:4561-4565.
39. Tan W, Wu JX, Zhang XM, et al. Associations of functional polymorphisms in cyclooxygenase-2 and platelet 12-lipoxygenase with risk of occurrence and advanced disease status of colorectal cancer. Carcinogenesis 2007;28:1197-1201.
40. Westbrook AM, Szakmary A, Schiestl RH. Mechanisms of intestinal inflammation and development of associated cancers: Lessons learned from mouse models. Mutat Res 2010;705:40-59.

Summary and future perspectives

Chapter 8

Summary

Infliximab is a monoclonal antibody against tumour necrosis factor α (TNF-α) and well incorporated in the treatment of patients with auto-inflammatory disorders such as inflammatory bowel disease (IBD). Blocking a cytokine central to the inflammatory response might lead to adverse events secondary to impaired immunity such as serious infections and even development of malignancies.
The primary aim of this thesis, as described in chapter 1, was to determine a safety profile of infliximab. To this end in **chapter 2A** we evaluated serious events, defined as any unfavourable event since the start of infliximab treatment, leading to hospitalization, malignancies and death, in a cohort of IBD patients. This cohort included 147 patients receiving 1924 infusions with infliximab (median of 10 per patient) over a median follow-up period of 59 months. Patients were followed for a total of 674 patient years. The rate of acute infusion reactions to infliximab (8%) seen was comparable with reported rates in other studies. Sixty-one percent of all patients were hospitalised after start of infliximab during follow-up. In 14% of all patients, the main reason for hospitalization was an infection which was considered at least possibly related to the use of infliximab. Nine patients developed malignancies and subgroup analysis, comparing patients with and without malignancies, showed a significant difference in duration of disease. A causal relationship between the use of infliximab and the development of solid tumours could not be made; the malignancies seem to be more related to underlying disease than to the use of infliximab. However, these malignancies developed at a relative young age. This indicates that careful monitoring of patients with IBD for signs which could be attributable to a malignancy, is necessary to detect serious events in an early stage.

We also commented on another study evaluating the long term safety of infliximab, in **chapter 2B**, and compared their results with other studies published in the same year (2009). Focusing on the development of malignancies, we emphasized the need of long term follow-up. A Belgian cohort study compared IBD patients treated with infliximab with patients not receiving any biological therapy and found no differences in terms of mortality, infection rates, and malignancies.[1] Infliximab therapy seems to be safe in the long term, although thorough follow-up evaluation of patients with IBD for the development of malignancies is warranted. This typically in patients receiving concomitant immunosuppressive therapy.[2]

Optimal monitoring of biological therapies is of major clinical importance. In clinical practice, monitoring vital signs (.e. blood pressure, pulse, temperature) is routinely performed with infliximab administration. This management strategy is based on the fact that infliximab may cause acute severe infusion reactions with associated signs and symptoms which most commonly include fever, chest pain, hypotension/ hypertension, or dyspnea.[3] In **chapter 2C** we evaluated the clinical value of a strategy that monitored vital signs. Scheduled monitoring of vital signs was of no value to predict the development of acute infusion reactions.

Therefore, scheduled measurement of vital signs during infliximab infusions is probably unnecessary.

In **chapter 3** we described the results of a multidisciplinary consensus meeting regarding current guidelines and consensus statements on the use of infliximab for auto-inflammatory disorders. Several topics (i.e. dosage of infliximab, monitoring of vital signs, use of concomitant medication and loss of response) were discussed and shortcomings in guidelines and consensus statements concerning these topics were identified. There are dosing schedules for different indications. National and internal guidelines lack advice how and when to choose for dosage adjustment or frequency intensification in case of loss of response. The high prevalence of co-morbidities in patients with auto inflammatory disorders, such as IBD,[4,5] plus the shared knowledge of specialists treating these patients with biological therapies, require a multi-disciplinary approach. In our current age of ongoing specialisation this is necessary to keep a multifaceted view on the use of biological agents like infliximab.

Infliximab-induced hepatotoxicity is reported in several case reports of IBD patients and a direct hepatotoxic effect has been suggested. In **chapter 4** we evaluated the in vitro toxicity of infliximab and compared this toxicity with the in vitro toxicity of immunosuppressants used in the treatment of IBD (thiopurines and methotrexate). There was no direct hepatotoxic effect of infliximab, even with increasing concentrations up to 5mg/ml.

The second part of this thesis dealt on identifying genetic susceptibility factors possibly associated with IBD. In **chapter 5** we tested the hypothesis that a functional polymorphism in *UGT1A1*, which is associated with hyperbilirubinemia, is associated with lower disease susceptibility to, and disease behavior within, IBD. Furthermore, we investigated a possible pharmacogenetic relationship between *UGT1A1*28* and thiopurine side effects. The cohort consisted of 751 patients (209 patients with ulcerative colitis 542 patients with Crohn's disease), and 930 healthy controls. Patients and controls were genotyped for the *UGT1A1*28* promoter polymorphism. Patients with Crohn's disease carried the *UGT1A1*28* homozygous genotype significantly less often compared to the control group, with an odds ratio of 0.64, 95% CI: 0.42-0.98. This suggests that the homozygous state of the *UGT1A1*28* polymorphism protects against Crohn's disease. We suggest that this results from the anti-oxidant capacity of bilirubin.

Dectin-1 is involved in the recognition of the fungal component β-glucan and amplifies pro-inflammatory cytokine production. An early stop codon polymorphism (c.714T>G) in *DECTIN-1* results in a loss-of-function (p.Y238X) and impaired cytokine responses. In patients with IBD, we observed that dectin-1 expression is elevated on macrophages, neutrophils, and other immune cells involved in the inflammatory reaction in IBD, as described in **chapter 6**.

We additionally genotyped genomic DNA of 778 patients with Crohn's disease, 759 patients with ulcerative colitis and 772 healthy controls for the c.714T>G polymorphism. However, no statistically significant difference in *DECTIN-1* c.714T>G allele frequencies was observed between IBD patients and healthy controls.

Cyclooxygenase-2 (COX-2) is a key enzyme involved in the conversion of arachidonic acid into prostaglandins and mainly induced at sites of inflammation in response to pro-inflammatory cytokines. Patients with IBD show increased expression of COX-2 in the gastrointestinal tract, which has also been observed in gastrointestinal adenocarcinomas and in ulcerative colitis-associated neoplasia. In **chapter 7** we determined the possible modulating effect of the functional *COX-2* -1195 A→G and -765G→C polymorphisms on the risk of IBD. A recent study from Denmark indicated that carriers of the *COX-2* -1195 A→G variant allele had increased risk of ulcerative colitis.[6] In our cohort, no such an effect could be seen. However, the -765GG genotype was more prevalent in patients with Crohn's disease compared to controls with an odds ratio (OR) of 1.33 (95% CI 1.04-1.69, $p < 0.05$). Efforts should be made to unravel the role of COX-2 and its derived prostaglandins in the pathogenesis of IBD.

Future perspectives

Safety assessment of therapies in inflammatory bowel disease

Although randomized controlled trails (RCTs) are the golden standards for studying efficacy, in studying safety aspects of medical treatment their use is limited by strict inclusion criteria and relatively short follow-up periods.[7] The ongoing long-term follow-up via registries is important in this respect.[8] Recently, the European League Against Rheumatism reported on elements to consider when establishing, analyzing and reporting safety data of biologics registers in rheumatology.[9] Most of these points also apply for safety analysis in patients with IBD. First of all, sample size is of major importance. Safety assessment requires large cohorts due to the relatively low background rates of serious adverse events. Furthermore, there should be clear reporting of study design and methodological techniques in order to be able to compare the results of these registries. A meaningful comparator (i.e. an unexposed cohort) must be included, which should be as similar as possible to the exposed group and potential biases should be noticed. Potential bias includes the higher rate of co-morbidity observed in referral centers together with the fact that clinical decisions will influence which patients receive more extensive treatment, potentially introducing 'channeling bias'.

The Dutch 'Parelsnoer' project, a biobank project in which all eight medical university centers in the Netherlands participate, might function as a template for a large clinical practice registry which enables to study long term safety of various treatments given in IBD. In this biobank project, standardized clinical data of IBD patients is obtained prospectively and integrated with standard molecular data from biomaterials taken.

This approach will enable us to collect a large dataset on patients with distinct clinical characteristics.

Predictors of effects and adverse events

The treatment of patients with IBD can be very challenging. First of all, although the natural history of IBD progressively leads to the development of complications in approximately two-thirds of Crohn's disease patients and less than one-third of ulcerative colitis patients, the clinical course of the disease varies greatly among patients.[10] Therefore, sensitive and specific markers to predict disease course and to identify those patients with an aggressive and progressive disease course that would benefit from early use of biologics to prevent future complication and surgery are necessary. Some clinical factors have been associated with a disabling course of disease including disease location (terminal ileum), disease behavior (structuring and penetrating behavior), age at diagnosis (< 40 years), the presence of perianal disease and the initial requirements for steroids.[11,12] Furthermore, several predictive factors for successful response to anti TNF therapy in Crohn's disease have been identified. Early luminal Crohn's disease, a high CRP, and high trough concentrations of infliximab have been associated with more durable maintenance of clinical response.[13] On the other hand, the presence of antibodies to infliximab (ATI) as a marker of immunogenicity, have been associated with an increased risk of infusion reactions and a reduced duration of response.[14-16] These observations however, have been challenged in a systematic review stating that a more relevant measure than ATIs is likely to be measurement of circulating drug concentration since measurement of ATIs is so dependent on analytical technique, timing of sampling, dosing regimen, circulating drug, concomitant therapy, and the individual patient.[17] Additionally, advanced age appears to be an independent risk factor for severe infections and mortality in patients given anti-tumor necrosis factor therapy for IBD.[18]

The use of a large clinical registry as mentioned before, would be of major importance to identify risk factors for serious outcomes in drug treatment. Since biological therapies are indicated for various auto inflammatory disorders, this identification process will require a multidisciplinary approach. Currently, the 'Parelsnoer' project includes a registry on IBD as well on rheumatoid arthritis. Since biomaterials are also obtained in this project, efforts should be made to unravel genetic susceptibility factors for efficacy and/ or side effects of biological treatment for various indications. In the future, this might enable clinicians to select the appropriate therapy for patients and to predict responses to these therapies.

Genetic susceptibility and IBD

In our studies we used a mechanistic approach, in which known functional polymorphisms were tested in IBD patients. However, in the era of genome wide association studies (GWAS), many new genomic regions containing IBD risk factors have been identified with much more statistical power. Knowledge on the functional

consequences of these genetic variants however is scarce. Additional studies are therefore needed to investigate the biological and cellular pathways involved.

Most of the alleles identified by GWAS are relatively common (allele frequencies >5%) and all these loci together explain less than 25% of the overall predicted genetic heritability in Crohn's disease.[19] Polymorphisms that have a stronger effect on disease development might be maintained at a lower frequency in the population (uncommon alleles) through negative selection, because they reduce reproductive fitness. The identification of these uncommon alleles might be greatly facilitated by new techniques as whole-exome and whole-genome sequencing. Recently, successful clinical application of whole-exome sequencing was shown in a child with intractable IBD, having a hemizygous missense mutation in the X-linked inhibitor of apoptosis gene.[20] These new sequencing technologies can also be used to catalogue the resident microflora at distinct body sites, and studying correlations between specific diseases and the composition of the microbiome.[21]

Furthermore, a full understanding of IBD requires capturing much of the genetic variation across the human population (i.e. genetic studies on IBD should also include non Caucasian populations), which will increase the power of GWAS. Also, the annotation and correlation of genomic information with high-quality phenotypic data is of major importance.[22] In the (near) future, this might lead to personalized medicine, tailored to individual patients.

Finally, to study the links between biological and environmental factors in complex diseases, such as IBD, it will be necessary to conduct suitably large (several hundred thousand people) prospective cohort studies with GWA genotyping, and reproducible reliable exposure measures at baseline.[22,23] Although the costs are high, several prospective cohorts for studying complex diseases have been established,[24-26] which will definitely enhance our understanding of the gene-environmental interplay.

References

1. Fidder H, Schnitzler F, Ferrante M, et al. Long-Term Safety of Infliximab for the treatment of Inflammatory Bowel Disease: A Single Center Cohort Study. Gut 2009;58:501-508.
2. Hoentjen F, van Bodegraven AA. Safety of anti-tumor necrosis factor therapy in inflammatory bowel disease. World J Gastroenterol 2009;15:2067-2073.
3. Cheifetz A, Smedley M, Martin S, et al. The incidence and management of infusion reactions to infliximab: a large center experience. Am J Gastroenterol. 2003;98:1315-1324.
4. Cooper GS, Bynum ML, Somers EC. Recent insights in the epidemiology of autoimmune diseases: Improved prevalence estimates and understanding of clustering of diseases. J Autoimmun. 2009;33:197-207.
5. Cohen R, Robinson D Jr, Paramore C, et al. Autoimmune disease concomitance among inflammatory bowel disease patients in the United States, 2001-2002. Inflamm Bowel Dis. 2008;14:738-743.
6. Andersen V, Nimmo E, Krarup HB, et al. Cyclooxygenase-2 (COX-2) polymorphisms and risk of inflammatory bowel disease in a Scottish and Danish case-control study. Inflamm Bowel Dis. 2011;17:937-946.
7. Solomon DH, Mercer E, Kavanaugh A. Observational studies on the risk of cancer associated with TNF-Inhibitors in RA: A review of their methodologies and results. Arthritis Rheum. 2011 [epub].
8. Askling J, Fahrbach K, Nordstrom B, et al. Cancer risk with tumor necrosis factor alpha (TNF) inhibitors: meta-analysis of randomized controlled trials of adalimumab, etanercept, and infliximab using patient level data. Pharmacoepidemiol Drug Saf. 2011;20:119-130.
9. Dixon WG, Carmona L, Finckh A, et al. EULAR points to consider when establishing, analysing and reporting safety data of biologics registers in rheumatology. Ann Rheum Dis. 2010;69:1596-1602.
10. Louis E, Belaiche J, Reenaers C. Do clinical factors help to predict disease course in inflammatory bowel disease? World J Gastroenterol. 2010;16:2600-2603.
11. Solberg IC, Vatn MH, Høie O, et al. Clinical course in Crohn's disease: results of a Norwegian population-based ten-year follow-up study. Clin Gastroenterol Hepatol 2007;5:1430-1438.
12. Beaugerie L, Seksik P, Nion-Larmurier I, et al. Predictors of Crohn's disease. Gastroenterology. 2006;130:650-656.
13. D'Haens GR, Panaccione R, Higgins PD, et al. The London Position Statement of the World Congress of Gastroenterology on Biological Therapy for IBD with the European Crohn's and Colitis Organization: when to start, when to stop, which drug to choose, and how to predict response? Am J Gastroenterol. 2011;106:199-212.
14. Hanauer SB, Wagner CL, Bala M, et al. Incidence and importance of antibody responses to infliximab after maintenance or episodic treatment in Crohn's disease. Clin Gastroenterol Hepatol. 2004;2:542-553.
15. Baert F , Noman M , Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease . N Engl J Med 2003;348:601-608.
16. Afif W, Loftus EV Jr, Faubion WA, et al. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. Am J Gastroenterol. 2010;105:1133-1139.
17. Cassinotti A, Travis S. Incidence and clinical significance of immunogenicity to infliximab in Crohn's disease: A critical systematic review. Inflamm Bowel Dis 2009;15:1264-1275.
18. Cottone M, Kohn A, Daperno M, et al. Advanced age is an independent risk factor for severe infections and mortality in patients given anti-tumor necrosis factor therapy for inflammatory bowel disease Clin Gastroenterol Hepatol. 2011;9:30-5.

19. Cho JH, Brant SR. Recent insights into the genetics of inflammatory bowel disease. Gastroenterology. 2011;140:1704-1712.
20. Worthey EA, Mayer AN, Syverson GD, et al. Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. Genet Med. 2011;13:255-262.
21. Green ED, Guyer MS, Manolio TA, et al. Charting a course for genomic medicine from base pairs to bedside. Nature. 2011;470:204-213.
22. Collins FS. The case for a US prospective cohort study of genes and environment. Nature. 2004;429:475-477.
23. Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. Nature. 2009;461:747-753.
24. UK Biobank. More information on http://www.ukbiobank.ac.uk/
25. The National Children's Study (USA). More information on: http://www.nationalchildrensstudy.gov
26. Triendl, R. Japan launches controversial Biobank project. Nature Med 2003; 9:982.

Chapter 9

Samenvatting en toekomstperspectieven

Samenvatting

Infliximab is een monoklonaal antilichaam gericht tegen tumor necrosis factor alpha (TNF-α). Het middel speelt een belangrijke rol in de behandeling van patiënten met auto-inflammatoire ziekten, zoals inflammatoire darmziekten (IBD). Het blokkeren van een belangrijk cytokine voor de inflammatoire respons kan leiden tot bijwerkingen die secundair zijn aan een verminderde weerstand, zoals ernstige infecties en zelfs het ontwikkelen van kwaadaardige tumoren (maligniteiten). Het primaire doel van dit proefschrift, zoals beschreven in **hoofdstuk 1**, was om het veiligheidsprofiel van infliximab te bepalen. In verband hiermee hebben we in **hoofdstuk 2A** gekeken naar de incidentie van ernstige neveneffecten, gedefinieerd als ongunstige gebeurtenissen die optraden na start van de behandeling met infliximab, welke leiden tot ziekenhuisopname, maligniteiten en overlijden binnen een cohort van IBD patiënten. Dit cohort betrof 147 patiënten die in totaal 1924 infusies met infliximab ontvingen (mediaan van 10 per patiënt) gedurende een mediane follow-up duur van 59 maanden. In totaal werden deze patiënten voor 674 patiëntjaren gevolgd. Het percentage acute infusiereacties op infliximab (8% van de patiënten) was vergelijkbaar met gerapporteerde percentages in andere studies. Gedurende de follow-up duur werd 61% van alle patiënten tenminste eenmaal opgenomen in het ziekenhuis. Bij 14% van alle patiënten was de belangrijkste reden voor ziekenhuisopname een infectie die beschouwd werd als ten minste mogelijk gerelateerd aan het gebruik van infliximab. Negen patiënten ontwikkelden maligniteiten in de follow-up periode en nadere analyse van patiënten met en zonder maligniteiten toonde een significant verschil in ziekteduur. Een causaal verband tussen het gebruik van infliximab en de ontwikkeling van maligniteiten kon niet worden gelegd. De maligniteiten lijken meer gerelateerd te zijn aan de onderliggende ziekte dan aan het gebruik van de infliximab. Echter, patiënten ontwikkelden deze maligniteiten op een relatief jonge leeftijd. Dit betekent dat zorgvuldige controle van IBD patiënten op tekenen die kunnen worden toegeschreven aan een maligniteit nodig is om dit in een vroeg stadium te kunnen detecteren.

In **hoofdstuk 2B** hebben we commentaar gegeven op een andere studie die de langetermijnveiligheid van infliximab heeft geëvalueerd en hebben we de resultaten vergeleken met andere studies die in hetzelfde jaar gepubliceerd zijn (2009). Met betrekking tot de ontwikkeling van maligniteiten benadrukken we de noodzaak van lange termijn follow-up. Een Belgische cohort studie vergeleek IBD patiënten die behandeld waren met infliximab met patiënten die geen behandeling met biologicals hadden ontvangen en vond geen verschillen in termen van sterfte, infecties en maligniteiten.[1] Hoewel infliximab een veilige en effectieve therapie lijkt te zijn op de lange termijn, is grondige follow-up met betrekking tot de ontwikkeling van maligniteiten noodzakelijk bij patiënten met IBD die behandeld worden met immuun modulerende therapie. Dit is vooral het geval bij patiënten die gelijktijdig meerdere vormen van immunosuppressieve therapie ontvangen.[2]

Optimale monitoring van de therapieën met biologicals is van klinisch belang. In de klinische praktijk is het controleren van de vitale functies (bloeddruk, pols, temperatuur) geïmplementeerd tijdens het toedienen van infliximab. Deze strategie is gebaseerd op het feit dat infliximab acute ernstige infuusreacties kan veroorzaken, vaak gekenmerkt door koorts, pijn op de borst, hypotensie of hypertensie en kortademigheid.[3] In **hoofdstuk 2C** hebben we de klinische relevantie van een strategie die de vitale functies controleert geëvalueerd. Geplande bewaking van de vitale functies is niet van waarde om de ontwikkeling van acute infusiereacties te voorspellen. Daarom is het routinematig meten van de vitale functies tijdens infliximabinfusies niet zinvol.

In **hoofdstuk 3** hebben we de resultaten van een multidisciplinaire consensusbijeenkomst over de huidige richtlijnen en consensus statements omtrent het gebruik van infliximab bij auto inflammatoire ziekten beschreven. Verschillende onderwerpen (waaronder; dosering van infliximab, monitoring van vitale functies, het gebruik van co-medicatie en het beleid bij verlies van de respons op infliximab) werden besproken en tekortkomingen in de huidige richtlijnen en consensus statements met betrekking tot deze onderwerpen werden geïdentificeerd. De verschillende indicaties omvatten verschillende doseringsschema's. In de nationale en interne richtlijnen is er weinig advies over hoe en wanneer men dient te kiezen voor aanpassing van de dosering of frequentie intensivering in geval van verlies van respons. De hoge prevalentie van comorbiditeit bij patiënten met auto inflammatoire ziekten zoals IBD,[4,5] plus de gezamenlijke kennis van specialisten aangaande de behandeling van deze patiënten met biologische therapieën, vereist een multidisciplinaire aanpak. In onze huidige tijd van voortdurende specialisatie is het noodzakelijk om een brede blik op het gebruik van biologicals zoals infliximab te houden.

Een aantal case reports maken melding van infliximab geïnduceerde hepatotoxiciteit bij patiënten met IBD en een direct hepatotoxisch effect van infliximab is verondersteld. In **hoofdstuk 4** hebben we de in vitro toxiciteit van infliximab onderzocht en vergeleken met de in vitro toxiciteit van immunosuppressiva zoals gebruikt bij de behandeling van IBD (thiopurines en methotrexaat). Geen direct hepatotoxische effect van infliximab werd waargenomen, zelfs niet bij concentraties tot 5 g / l.

In het tweede deel van dit proefschrift hebben we getracht genetische risico factoren die mogelijk geassocieerd zijn met inflammatoire darmziekten te identificeren. Daarbij hebben wij ons gericht op functionele polymorfismen en hebben we hun potentiële rol in de pathogenese van IBD onderzocht. In **hoofdstuk 5** testten wij de hypothese dat een functioneel polymorfisme in *UGT1A1*, dat wordt geassocieerd met hyperbilirubinemie, geassocieerd is met een lager risico op, en ander ziektegedrag binnen, IBD. Verder onderzochten we een mogelijke farmacogenetische relatie tussen *UGT1A1*28* en thiopurine bijwerkingen. Het cohort bestond uit 751

patiënten (209 patiënten met colitis ulcerosa en 542 patiënten met de ziekte van Crohn) en 930 gezonde controles. Patiënten en controles werden gegenotypeerd voor het *UGT1A1*28* promoter polymorfisme. Binnen het cohort van patiënten met de ziekte van Crohn waren er significant minder dragers van het *UGT1A1*28* homozygote genotype in vergelijking met de controlegroep met een odds ratio van 0.64, 95% betrouwbaarheidsinterval: 0.42-0.98. Dit suggereert dat de homozygote toestand van het *UGT1A1*28* polymorfisme beschermt tegen de ziekte van Crohn. Mogelijk vloeit dit voort uit de antioxidantcapaciteit van bilirubine.

Dectine-1 is betrokken bij de herkenning van de schimmelcomponent β-glucan en versterkt de pro-inflammatoire cytokine productie. Een stop codon polymorfisme (C.714T>G) in *DECTINE-1* leidt tot een verlies van functie (p.Y238X) en verminderde cytokine productie. Bij patiënten met inflammatoire darmziekten hebben we vastgesteld dat de dectine-1 expressie verhoogd is in macrofagen, neutrofielen en andere immuun cellen die betrokken zijn bij de ontstekingsreactie, zoals beschreven in **hoofdstuk 6**. Verder hebben wij het DNA van 778 patiënten met de ziekte van Crohn, 759 patiënten met colitis ulcerosa en 772 gezonde controles gegenotypeerd voor het c.714T>G polymorfisme. Er werd echter geen statistisch significant verschil in *DECTINE-1* c.714T>G allel frequenties waargenomen tussen IBD patiënten en gezonde controles.

Cyclooxygenase-2 (COX-2) is een belangrijk enzym dat betrokken is bij de omzetting van arachidonzuur in prostaglandines. COX-2 wordt vooral geïnduceerd in ontstekingsgebieden in reactie op pro-inflammatoire cytokines. Patiënten met IBD vertonen een verhoogde expressie van COX-2 in het maag-darmkanaal en dit is tevens waargenomen in gastro-intestinale adenocarcinomen en colitis ulcerosa geassocieerde neoplasie. In **hoofdstuk 7** hebben we het mogelijke modulerende effect van de functionele *COX-2* -1195 A → G en -765G → C polymorfismen op het risico op inflammatoire darmziekten bepaald. Een recente studie uit Denemarken gaf aan dat dragers van de *COX-2* -1195 A → G allel variant een verhoogd risico hebben op colitis ulcerosa.[6] In ons cohort zagen wij een dergelijke effect niet. Echter, het -765GG genotype werd vaker gezien bij patiënten met de ziekte van Crohn in vergelijking met gezonde controles met een odds ratio van 1.33 (95% betrouwbaarheidsinterval: 1.04-1.69, p <0.05). Verder onderzoek naar de rol van COX-2 en de daarvan afgeleide prostaglandines in de pathogenese van IBD is noodzakelijk.

Toekomstperspectieven

Veiligheidsanalyse van therapieën voor inflammatoire darmziekten

Hoewel gerandomiseerde gecontroleerde trails (RCT's) de gouden standaard zijn voor het bestuderen van de effectiviteit van een geneesmiddel, zijn deze studies door strikte inclusiecriteria en de relatief korte follow-up periodes beperkt bruikbaar voor het bestuderen van de veiligheid van geneesmiddelen.[7] Met betrekking tot het bestuderen van de veiligheid zijn langdurende follow-up databases van belang.

Recent heeft de European League Against Rheumatism gerapporteerd welke elementen men dient te overwegen bij het vaststellen, analyseren en rapporteren van de veiligheid van biologicals binnen deze databases in de reumatologie.[9] De meeste van deze punten zijn ook van toepassing voor de analyse van de veiligheid van biologicals bij patiënten met IBD. Allereerst is de steekproefomvang van groot belang. Veiligheidsbeoordeling vereist grote cohorten door de relatief lage incidentie van ernstige bijwerkingen. Bovendien moet er een duidelijke rapportage zijn van de onderzoeksopzet en methodologische technieken om de resultaten van deze databases te kunnen vergelijken. Eveneens dient er een patiëntencohort ter vergelijking te worden opgenomen (dat wil zeggen een cohort patiënten dat het middel niet heeft gekregen), welke zoveel mogelijk gelijk is aan de behandelde groep. Mogelijke vormen van 'bias' dienen te worden opgemerkt. Potentiële bias omvat de verhoogde co-morbiditeit zoals waargenomen in verwijscentra, alsmede het feit dat de klinische toestand beïnvloedt welke patiënten een uitgebreidere behandeling krijgen, waardoor mogelijk 'channeling bias' wordt ingevoerd.

Het Nederlandse 'Parelsnoerproject', een biobankproject waaraan alle acht medische universitaire centra in Nederland deelnemen, kan fungeren als een sjabloon voor een grote klinische praktijkdatabase die het mogelijk maakt om de langetermijnveiligheid van de gegeven verschillende behandelingen van IBD te bestuderen. In dit biobankproject worden gestandaardiseerde klinische gegevens van IBD patiënten prospectief verkregen en geïntegreerd met moleculaire gegevens die afkomstig zijn van de afgenomen biomaterialen. Deze aanpak zal ons in staat stellen een grote dataset van patiënten te verzamelen met duidelijke klinische kenmerken.

Voorspellers van effecten en bijwerkingen

De behandeling van patiënten met inflammatoire darmziekten kan zeer uitdagend zijn. Hoewel het natuurlijk beloop van IBD geleidelijk leidt tot de ontwikkeling van complicaties bij ongeveer tweederde van patiënten met de ziekte van Crohn en bij minder dan een derde van patiënten met colitis ulcerosa, varieert het klinische verloop van de ziekte sterk.[10] Daarom zijn sensitieve en specifieke markers noodzakelijk om het ziekteverloop te kunnen voorspellen en om patiënten met een agressieve en progressieve ziekte te identificeren. Deze patiënten zouden kunnen profiteren van een vroeg gebruik van biologicals om toekomstige complicaties en chirurgie te voorkomen. Een aantal klinische factoren is geassocieerd met een gecompliceerd beloop van de ziekte, zoals ziektelocatie (terminale ileum), ziektegedrag (stricturen en fistels), leeftijd bij het stellen van de diagnose (<40 jaar), de aanwezigheid van perianale ziekte en vroege noodzakelijkheid van corticosteroiden.[11, 12] Daarnaast zijn er verschillende voorspellende factoren voor een succesvolle reactie op de anti TNF therapie bij de ziekte van Crohn geïdentificeerd. Vroege luminale ziekte van Crohn, een hoog CRP en hoge infliximab dalconcentraties zijn geassocieerd met een langdurige klinische respons.[13] Aan de andere kant is de aanwezigheid van antilichamen tegen infliximab (ATI) geassocieerd met een verhoogd risico op

infusiereacties en een verminderde responsduur.[14-16] Deze waarnemingen zijn echter betwist in een systematische review. Hierin werd gesteld dat het bepalen van circulerende geneesmiddel-concentraties zeer waarschijnlijk relevanter is dan ATI aangezien de meting van ATI sterk afhankelijk is van de analyse techniek, het moment van monsterafname, de dosering, het circulerende geneesmiddel, de gelijktijdige behandeling met andere geneesmiddelen en de individuele patient.[17] Verder blijkt gevorderde leeftijd nog een onafhankelijke risicofactor te zijn voor ernstige infecties en mortaliteit bij patiënten met inflammatoire darmziekten die behandeld worden met anti-TNF therapie.[18]

Zoals eerder vermeld is het gebruik van een grote klinische database van groot belang om risico's op ernstige gevolgen bij behandeling met geneesmiddelen te identificeren. Aangezien de biologische therapieën zijn geïndiceerd voor diverse auto inflammatoire aandoeningen, zal dit identificatieproces een multidisciplinaire aanpak vereisen. Op dit moment omvat het Parelsnoerproject zowel een database voor inflammatoire darmziekten als voor reumatoïde artritis. Omdat in dit project tevens biomaterialen worden verkregen, moeten er inspanningen worden geleverd om voor de verschillende indicaties genetische factoren te ontdekken die de werkzaamheid en/of bijwerkingen van de biologische behandeling beïnvloeden. Dit zal het voor clinici in de toekomst mogelijk maken om de juiste therapie voor de juiste patiënt te selecteren en zal hen helpen om reacties op deze therapieën te voorspellen.

Genetische vatbaarheid en IBD

In onze studies hebben wij gebruik gemaakt van een mechanistische benadering, waarbij bekende functionele polymorfismen bepaald zijn bij patiënten met IBD. Echter, in het huidige tijdperk van genoom wijde associatie studies (GWAS) zijn er veel nieuwe IBD risico gengebieden geïdentificeerd met een veel grotere mate van statistische zekerheid. Kennis van de functionele gevolgen van deze genetische varianten is echter schaars. Aanvullende studies zijn daarom nodig om de betrokken cellulaire en biologische mechanismen te onderzoeken.

De meeste allelen die door GWAS geïdentificeerd zijn komen relatief vaak voor (allel frequenties > 5%) en al deze loci samen verklaren minder dan 25% van de totaal voorspelde genetische heritabiliteit van de ziekte van Crohn.[19] Polymorfismen die een groter effect hebben op ziekteontwikkeling kunnen door negatieve selectie echter in een lagere frequentie in de bevolking voorkomen (zeldzame allelen) doordat ze de reproductiviteit verminderen. De identificatie van deze zeldzame allelen zou in hoge mate worden vergemakkelijkt door nieuwe technieken als exoom sequencing en het sequencen van het volledig genoom. Recent was er een succesvolle klinische toepassing van exoom sequencing bij een kind met onbehandelbare IBD die een hemizygote missense mutatie in het X-linked inhibitor of apoptosis gen bleek te hebben.[20] Deze nieuwe sequencing technieken kunnen ook gebruikt worden om de residentiële microflora van de verschillende lichaamsplekken te identificeren en

op deze wijze de correlaties tussen specifieke ziekte en de compositie van het microbioom te bestuderen.[21] Verder is het voor een volledig begrip van IBD vereist om de genetische variatie binnen de menselijk populatie te bepalen, wat inhoudt dat bij genetische studies binnen IBD ook andere rassen dan het Kaukasische ras geïncludeerd moeten worden om zo de power van GWAS te vergroten. Ook het beschrijven en correleren van genetische informatie aan gedetailleerde fenotypische data is van groot belang.[22] In de (nabije) toekomst kan dit leiden tot 'personalized medicine', toegespitst op de individuele patiënt.

Ten slotte is het voor het bestuderen van de relaties tussen biologische- en omgevingsfactoren binnen complexe ziekten, zoals IBD, nodig om grote prospectieve cohort studies (enkele honderdduizenden mensen) uit te voeren met GWA genotypering en reproduceerbare betrouwbare expositie op baseline.[22, 23] Ondanks de hoge kosten zijn er al verscheidene prospectieve cohort studies opgezet om complexe ziekten te bestuderen.[24-26] Deze studies zullen absoluut ons begrip van gen-milieu interactie vergroten.

Referenties

1. Fidder H, Schnitzler F, Ferrante M, et al. Long-Term Safety of Infliximab for the treatment of Inflammatory Bowel Disease: A Single Center Cohort Study. Gut 2009;58:501-508.
2. Hoentjen F, van Bodegraven AA. Safety of anti-tumor necrosis factor therapy in inflammatory bowel disease. World J Gastroenterol 2009;15:2067-2073.
3. Cheifetz A, Smedley M, Martin S, et al. The incidence and management of infusion reactions to infliximab: a large center experience. Am J Gastroenterol. 2003;98:1315-1324.
4. Cooper GS, Bynum ML, Somers EC. Recent insights in the epidemiology of autoimmune diseases: Improved prevalence estimates and understanding of clustering of diseases. J Autoimmun. 2009;33:197-207.
5. Cohen R, Robinson D Jr, Paramore C, et al. Autoimmune disease concomitance among inflammatory bowel disease patients in the United States, 2001-2002. Inflamm Bowel Dis. 2008;14:738-743.
6. Andersen V, Nimmo E, Krarup HB, et al. Cyclooxygenase-2 (COX-2) polymorphisms and risk of inflammatory bowel disease in a Scottish and Danish case-control study. Inflamm Bowel Dis. 2011;17:937-946.
7. Solomon DH, Mercer E, Kavanaugh A. Observational studies on the risk of cancer associated with TNF-Inhibitors in RA: A review of their methodologies and results. Arthritis Rheum. 2011 [epub].
8. Askling J, Fahrbach K, Nordstrom B, et al. Cancer risk with tumor necrosis factor alpha (TNF) inhibitors: meta-analysis of randomized controlled trials of adalimumab, etanercept, and infliximab using patient level data. Pharmacoepidemiol Drug Saf. 2011;20:119-130.
9. Dixon WG, Carmona L, Finckh A, et al. EULAR points to consider when establishing, analysing and reporting safety data of biologics registers in rheumatology. Ann Rheum Dis. 2010;69:1596-1602.
10. Louis E, Belaiche J, Reenaers C. Do clinical factors help to predict disease course in inflammatory bowel disease? World J Gastroenterol. 2010;16:2600-2603.
11. Solberg IC, Vatn MH, Høie O, et al. Clinical course in Crohn's disease: results of a Norwegian population-based ten-year follow-up study. Clin Gastroenterol Hepatol 2007;5:1430-1438.
12. Beaugerie L, Seksik P, Nion-Larmurier I, et al. Predictors of Crohn's disease. Gastroenterology. 2006;130:650-656.
13. D'Haens GR, Panaccione R, Higgins PD, et al. The London Position Statement of the World Congress of Gastroenterology on Biological Therapy for IBD with the European Crohn's and Colitis Organization: when to start, when to stop, which drug to choose, and how to predict response? Am J Gastroenterol. 2011;106:199-212.
14. Hanauer SB, Wagner CL, Bala M, et al. Incidence and importance of antibody responses to infliximab after maintenance or episodic treatment in Crohn's disease. Clin Gastroenterol Hepatol. 2004;2:542-553.
15. Baert F , Noman M , Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease . N Engl J Med 2003;348:601-608.
16. Afif W, Loftus EV Jr, Faubion WA, et al. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. Am J Gastroenterol. 2010;105:1133-1139.
17. Cassinotti A, Travis S. Incidence and clinical significance of immunogenicity to infliximab in Crohn's disease: A critical systematic review. Inflamm Bowel Dis 2009;15:1264-1275.
18. Cottone M, Kohn A, Daperno M, et al. Advanced age is an independent risk factor for severe infections and mortality in patients given anti-tumor necrosis factor therapy for inflammatory bowel disease Clin Gastroenterol Hepatol. 2011;9:30-5.

19. Cho JH, Brant SR. Recent insights into the genetics of inflammatory bowel disease. Gastroenterology. 2011;140:1704-1712.
20. Worthey EA, Mayer AN, Syverson GD, et al. Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. Genet Med. 2011;13:255-262.
21. Green ED, Guyer MS, Manolio TA, et al. Charting a course for genomic medicine from base pairs to bedside. Nature. 2011;470:204-213.
22. Collins FS. The case for a US prospective cohort study of genes and environment. Nature. 2004;429:475-477.
23. Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. Nature. 2009;461:747-753.
24. UK Biobank. More information on http://www.ukbiobank.ac.uk/
25. The National Children's Study (USA). More information on: http://www.nationalchildrensstudy.gov
26. Triendl, R. Japan launches controversial Biobank project. Nature Med 2003; 9:982.

Dankwoord & Curriculum Vitae

Chapter 10

Dankwoord

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Hilbert de Vries werd op 11 september 1984 geboren te Veenendaal. Na het behalen van zijn VWO diploma in 2002, studeerde hij Werktuigbouwkunde aan de Universiteit Twente. Na het behalen van zijn propedeuse startte hij met de nieuwe opleiding Technische Geneeskunde aan dezelfde universiteit. Na het behalen van zijn propedeuse van deze opleiding werd in 2004 de overstap gemaakt naar de studie Geneeskunde aan de Radboud Universiteit Nijmegen. In 2007 deed hij in het kader van zijn wetenschappelijke stage een 6 maanden durend onderzoek aan de University of Alberta, Edmonton, Canada, naar de effecten van prebiotica (niet verteerbare voedingsvezels) op de mate van darmontsteking bij HLA-B27 transgene ratten (begeleider dr. L.A. Dieleman). In verband met de wachttijd die hij had voordat zijn coschappen begonnen, werkte hij vervolgens onder begeleiding van dr. D.J. de Jong als student-assistent op de afdeling Maag-darm-leverziekten van het UMC St Radboud. Daarmee werd een begin gemaakt aan het huidige promotieonderzoek (promotor prof. dr. J.P.H. Drenth). In 2011 trouwde hij met Evelien, studeerde hij af en werd tevens dit promotieonderzoek afgerond. Tijdens zijn co-schappen als ook tijdens zijn senior coschap in Biharamulo, Tanzania, kwam hij in aanraking met de chirurgie. Dit vakgebied wekte zijn bijzondere interesse op. Hij werkt nu als arts-assistent niet in opleiding bij de afdeling chirurgie van het St Antoniusziekenhuis te Nieuwegein (opleider dr. P.M.N.Y.H. Go).